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NITROGEN METABOLISM OF TWO-YEAR-OLD STEERS

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PLAN OF THE EXPERIMENT

The present paper is one of several reports on a digestion and metabolism experiment conducted at the Illinois Agricultural Experiment Station upon eight 2-year-old steers.[1] This paper reports the nitrogen balance of each steer as determined for each week during a total period of 37 weeks. The experiment was divided into five experimental periods. During the first period the ration consisted of clover hay and ground corn in equal amounts by weight; during the second, of one part of clover hay and three parts of ground corn; during the third, of one part of clover hay and five parts of ground corn; and during the fourth and fifth, of one part of clover hay, four parts of ground corn, and one part of linseed oil meal. The last proportion was maintained to the end of the experiment. These changes are comparable to the changes often made in the proportions of roughage and concentrates in ordinary feeding practice. The first experimental period was five weeks in length; the second, third, and fourth were each six weeks in length; and the fifth was four weeks in length. The changes in the ration made from one test period to another were effected very gradually in transitional periods, one of which immediately followed each experimental period. The first and third transitional periods were two weeks in length; the second and fourth were three weeks in length. Table I gives the experimental periods and the feeds given in each period.

[1] MUMFORD, H. W., GRINDLEY, H. S., HALL, L. D., EMMETT, A. D., and others. A STUDY OF THE DIGESTIBILITY OF RATIONS FOR STEERS. . . Ill. Agr. Exp. Sta. Bul. 177, p. 233–278, illus. 1914.

MUMFORD, H. W., GRINDLEY, H. S., EMMETT, A. D., and BULL, SLEETER. A STUDY OF THE RATE AND ECONOMY OF GAINS OF FATTENING STEERS. . . Ill. Agr. Exp. Sta. Bul. 197, p. 563–604. 1917.

GRINDLEY, H. S., MUMFORD, H. W., EMMETT, A. D., and BULL, SLEETER. FERTILIZING CONSTITUENTS EXCRETED BY 2-YEAR-OLD STEERS. Ill. Agr. Exp. Sta. Bul. 209, p. 127–162. 1918.

TABLE I.—*Ratios of hay, corn, and linseed meal in rations at different periods of the experiment*

Period No.	Weeks included in experiment.	Number of weeks in period.	Ratio of hay to corn to linseed meal.
1	1–5	5	1:1:0
2	8–13	6	1:3:0
3	17–22	6	1:5:0
4	25–30	6	1:4:1
5	34–37	4	1:4:1

To determine the effect of variations in the amount of feed consumed the eight steers were divided into four lots of two animals each, and each lot was given throughout the experiment an amount of feed different from that received by the other lots. The lots were as similar as possible in age, condition, and breeding. One lot was given just enough feed to maintain the weights of the steers about constant; another, as much as the steers would eat readily; another, an amount of feed equal to the maintenance ration plus one-third of the difference between the maintenance and the full feed rations; and another, an amount equal to the maintenance ration plus two-thirds of the difference between the maintenance and full feed rations. Beginning with the thirty-first week, one steer each from the maintenance, the one-third, and the two-thirds feed lots was gradually put on a full feed ration and kept upon it until the end of the experiment.

Tables II and III show the consumption of digestible crude protein and net energy per period. Table IV gives the weights of the steers. Table V gives the amounts of nitrogen consumed, the amounts of urinary nitrogen, fecal nitrogen, total excretory nitrogen, and the nitrogen balance of each steer per week.

TABLE II.—*Digestible crude protein consumed daily per 1,000 pounds live weight*

[Results expressed in pounds]

Period No.	Weeks included in experiment.	Ratio of hay to corn to oil-meal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.	
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.[a]	Steer 665.	Steer 663.[b]	Steer 661.
1	1–5	1:1:0	0.56	0.65	0.82	0.74	0.88	0.90	1.04	0.96
2	8–13	1:3:0	.44	.45	.67	.69	.81	.87	.85	.85
3	17–22	1:5:0	.40	.40	.54	.56	.60	.67	.66	.65
4	25–30	1:4:1	.83	.82	.99	1.00	1.12	1.20	1.19	1.30
5[c]	34–37	1:4:1	1.47	.77	1.34	.98	1.31	1.15	.....	1.34

[a] Removed at end of thirty-fourth week.
[b] Removed at end of thirtieth week.
[c] Steers 650, 666, and 652 were on full feed in period 5.

TABLE III.—*Net energy consumed daily per 1,000 pounds live weight* [a]

[Results expressed in therms]

Period No.	Weeks included in experiment.	Ratio of hay to corn to oilmeal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.	
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.[b]	Steer 665.	Steer 663.[c]	Steer 661.
1.....	1–5	1:1:0	6.98	7.13	9.46	8.64	11.05	11.17	12.69	12.43
2.....	8–13	1:3:0	6.95	7.00	9.80	9.77	12.25	12.59	13.67	13.20
3.....	17–22	1:5:0	6.73	6.73	8.87	9.35	11.16	11.55	11.53	10.75
4.....	25–30	1:4:1	6.59	6.47	8.37	8.41	9.90	10.86	10.52	12.22
5[d].....	34–37	1:4:1	12.28	6.03	11.49	6.74	11.27	9.88	.....	11.96

[a] Assuming that the energy requirements vary directly as the two-thirds power of the live weight.
[b] Removed at end of thirty-fourth week.
[c] Removed at end of thirtieth week.
[d] Steers 650, 666, and 652 were on full feed in period 5.

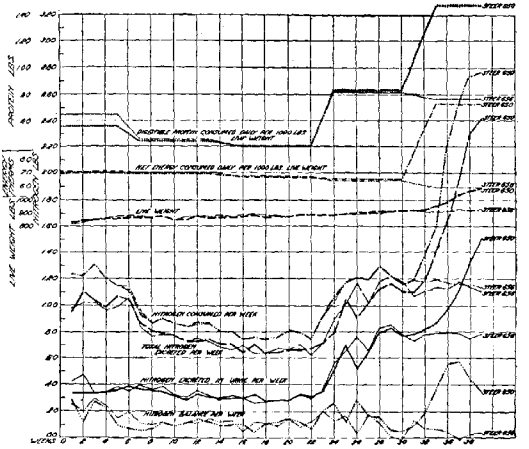

FIG. 1.—Nitrogen metabolism of steers in the maintenance lot.

RELATION OF NITROGEN CONSUMED TO NITROGEN EXCRETED

MAINTENANCE LOT.—Figure 1 shows the daily consumption of digestible crude protein per 1,000 pounds live weight during each period, the daily consumption of net energy per 1,000 pounds live weight during each period, the average live weight per week, the nitrogen consumed per week, the total nitrogen excreted per week, the urinary nitrogen per week, and the nitrogen balance per week of the two steers of the maintenance lot.

TABLE IV.—*Weights of steers at beginning and end of each period*

[Results expressed in pounds]

Period No.	Week on which test ended.	Ratio of hay to corn to oilmeal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.	
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.[a]	Steer 665.	Steer 663.[b]	Steer 661.
1.....	1	1:1:0	814	809	849	901	869	886	873	1,004
1.....	5	1:1:0	869	878	937	970	975	1,004	992	1,093
2.....	8	1:3:0	864	869	932	978	977	1,018	1,019	1,109
2.....	13	1:3:0	859	872	988	1,033	1,071	1,084	1,109	1,191
3.....	17	1:5:0	879	882	1,000	1,053	1,098	1,143	1,113	1,217
3.....	22	1:5:0	881	886	1,023	1,107	1,161	1,186	1,202	1,255
4.....	25	1:4:1	895	891	1,027	1,115	1,177	1,204	1,244	1,295
4.....	30	1:4:1	930	922	1,084	1,178	1,250	1,283	1,320	1,404
5[c]	34	1:4:1	972	912	1,138	1,195	1,275	1,309	.....	1,462
5.....	37	1:4:1	1,087	934	1,197	1,220	(1,286)	1,348	.....	1,518

[a] Removed at end of thirty-fourth week.
[b] Removed at end of thirtieth week.
[c] Steers 650, 667, 660, and 652 were on full feed in period 5.

TABLE V.—*Nitrogen metabolism of steers*

MAINTENANCE LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 630.					Steer 636.				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	1.231	0.329	0.620	0.949	0.282	1.231	0.425	0.559	0.984	0.247
2	1.217	.327	.771	1.098	.119	1.217	.468	.590	1.018	.199
3	1.304	.328	.708	1.036	.268	1.304	.328	.688	1.016	.288
4	1.203	.340	.646	.986	.217	1.203	.343	.622	.965	.238
5	1.145	.355	.703	1.058	.087	1.145	.382	.611	.993	.152
6	1.114	.378	.666	1.044	.070	1.142	.350	.597	.947	.195
7	.926	.361	.513	.874	.052	.947	.391	.446	.837	.110
8	.874	.353	.460	.813	.061	.874	.364	.408	.772	.102
9	.896	.341	.445	.786	.110	.896	.376	.405	.781	.115
10	.861	.310	.460	.770	.091	.861	.311	.459	.770	.091
11	.843	.279	.453	.732	.111	.843	.297	.431	.728	.115
12	.867	.321	.404	.725	.142	.860	.346	.405	.751	.109
13	.861	.296	.445	.741	.120	.861	.310	.409	.719	.142
14	.796	.294	.411	.705	.091	.796	.294	.383	.677	.119
15	.797	.308	.392	.700	.097	.797	.288	.357	.663	.134
16	.744	.286	.357	.643	.101	.744	.289	.403	.692	.052
17	.755	.316	.387	.703	.052	.755	.259	.375	.634	.121
18	.740	.266	.369	.635	.105	.740	.267	.364	.631	.109
19	.763	.276	.368	.644	.119	.763	.277	.391	.668	.095
20	.808	.276	.387	.663	.145	.808	.276	.389	.665	.143
21	.787	.319	.344	.663	.124	.787	.325	.377	.702	.085
22	.742	.314	.360	.674	.068	.742	.301	.316	.617	.125
23	.917	.353	.355	.708	.209	.917	.358	.355	.713	.204
24	1.056	.512	.283	.795	.261	1.027	.564	.332	.896	.131
25	1.175	.691	.358	1.049	.126	1.175	.651	.356	1.007	.168
26	1.205	.521	.393	.914	.291	1.205	.758	.411	1.169	.036
27	1.194	.640	.383	1.023	.171	1.194	.648	.370	1.018	.176
28	1.293	.795	.338	1.133	.160	1.293	.830	.348	1.178	.115
29	1.223	.834	.331	1.165	.058	1.223	.854	.364	1.218	.005
30	1.161	.767	.346	1.113	.048	1.161	.778	.401	1.179	−.018
31	1.194	.783	.317	1.100	.094	1.194	.732	.335	1.067	.127
32	1.385	.819	.376	1.195	.190	1.181	.781	.333	1.114	.067
33	1.792	.866	.549	1.415	.377	1.200	.792	.352	1.144	.056
34	2.200	.979	.666	1.645	.555	1.171	.787	.343	1.130	.041
35	2.526	1.132	.818	1.950	.576	1.158	.785	.395	1.180	−.022
36	2.742	1.340	.063	2.303	.439	1.162	.749	.389	1.138	.024
37	2.768	1.512	.903	2.415	.353	1.142	.793	.314	1.107	.035

TABLE V.—*Nitrogen metabolism of steers*—Continued

ONE-THIRD FEED LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 666.					Steer 669.				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	1.705	0.334	0.603	0.940	0.765	1.761	0.431	0.902	1.333	0.428
2	1.686	.427	.915	1.342	.344	1.741	.479	1.122	1.601	.140
3	1.806	.443	.962	1.405	.401	1.865	.479	.930	1.409	.456
4	1.722	.448	.962	1.410	.312	1.750	.566	.936	1.442	.308
5	1.626	.504	.974	1.478	.148	1.677	.566	1.013	1.579	.098
6	1.613	.482	.962	1.444	.169	1.714	.534	.823	1.357	.357
7	1.409	.553	.798	1.351	.058	1.481	.585	.801	1.386	.095
8	1.349	.537	.670	1.207	.142	1.406	.564	.727	1.291	.115
9	1.404	.542	.679	1.221	.183	1.462	.555	.675	1.230	.232
10	1.385	.486	.674	1.160	.225	1.439	.545	.660	1.205	.234
11	1.376	.579	.681	1.260	.116	1.421	.564	.660	1.224	.197
12	1.415	.571	.663	1.234	.181	1.480	.526	.654	1.180	.300
13	1.405	.564	.660	1.224	.181	1.480	.564	.653	1.217	.263
14	1.251	.563	.625	1.188	.063	1.371	.573	.679	1.246	.125
15	1.241	.510	.595	1.105	.136	1.374	.600	.579	1.179	.195
16	1.160	.469	.536	1.005	.155	1.287	.591	.565	1.156	.131
17	1.165	.474	.587	1.061	.104	1.294	.550	.573	1.123	.171
18	1.120	.500	.555	1.055	.065	1.226	.549	.587	1.136	.090
19	1.156	.457	.544	1.001	.155	1.243	.531	.589	1.120	.123
20	1.224	.396	.547	.943	.281	1.316	.570	.531	1.101	.215
21	1.192	.586	.546	1.132	.060	1.282	.559	.605	1.164	.118
22	1.060	.456	.489	.945	.115	1.145	.554	.506	1.060	.085
23	1.317	.591	.470	1.061	.256	1.425	.652	.565	1.217	.208
24	1.537	.538	.555	1.093	.444	1.659	.779	.631	1.410	.249
25	1.679	.540	.530	1.070	.609	1.813	1.125	.624	1.749	.064
26	1.721	.602	.598	1.200	.521	1.859	.904	.668	1.662	.197
27	1.706	.915	.631	1.546	.160	1.843	1.080	.620	1.700	.143
28	1.847	1.001	.510	1.511	.336	1.995	1.168	.577	1.745	.250
29	1.799	1.069	.652	1.721	.078	1.943	1.215	.576	1.791	.152
30	1.707	1.159	.563	1.722	—.015	1.843	1.219	.502	1.811	.032
31	1.773	1.151	.553	1.704	.069	1.954	1.195	.595	1.790	.164
32	1.958	1.137	.558	1.695	.263	1.932	1.225	.592	1.817	.115
33	2.374	1.284	.772	2.056	.318	1.964	1.210	.558	1.768	.196
34	2.625	1.418	.887	2.305	.320	1.916	1.225	.588	1.813	.103
35	2.741	1.603	.981	2.584	.157	1.894	1.208	.584	1.792	.102
36	2.863	1.682	1.043	2.725	.138	1.902	1.199	.573	1.772	.130
37	2.872	1.681	1.119	2.800	.072	1.868	1.224	.519	1.743	.125

TABLE V.—*Nitrogen metabolism of steers*—Continued

TWO-THIRDS FEED LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 652.[a]					Steer 665.				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1.....	2.202	0.438	1.276	1.714	0.488	2.286	0.454	1.340	1.794	0.492
2.....	2.180	.571	1.344	1.915	.265	2.265	.521	1.366	1.887	.378
3.....	2.336	.488	1.324	1.812	.524	2.429	.584	1.201	1.785	.644
4.....	2.212	.557	1.227	1.784	.428	2.298	.566	1.373	1.929	.359
5.....	2.114	.599	1.273	1.872	.242	2.209	.628	1.399	2.027	.182
6.....	2.116	.609	1.168	1.777	.339	2.268	.623	1.362	1.985	.283
7.....	1.896	.655	1.042	1.697	.199	1.881	.639	1.148	1.787	.094
8.....	1.824	.659	1.004	1.663	.161	1.934	.621	.958	1.579	.355
9.....	1.911	.680	.945	1.625	.286	2.028	.644	1.012	1.656	.372
10.....	1.906	.597	.962	1.559	.347	2.019	.724	1.007	1.731	.288
11.....	1.894	.667	.987	1.654	.240	2.013	.670	1.047	1.717	.296
12.....	1.948	.616	.939	1.555	.393	2.096	.686	.974	1.660	.436
13.....	1.934	.629	1.033	1.662	.272	2.114	.719	1.003	1.722	.392
14.....	1.710	.630	.886	1.516	.194	1.953	.730	1.018	1.748	.205
15.....	1.686	.577	.827	1.404	.282	1.821	.643	.962	1.605	.216
16.....	1.578	.555	.796	1.351	.227	1.834	.650	1.031	1.681	.153
17.....	1.575	.536	.802	1.338	.237	1.833	.686	.961	1.647	.186
18.....	1.522	.536	.841	1.377	.145	1.723	.649	.896	1.545	.178
19.....	1.570	.561	.839	1.400	.170	1.745	.636	.903	1.539	.206
20.....	1.662	.489	.838	1.327	.335	1.847	.672	.853	1.525	.322
21.....	1.619	.630	.755	1.385	.234	1.799	.729	.792	1.521	.278
22.....	1.378	.656	.647	1.303	.075	1.547	.621	.807	1.428	.119
23.....	1.719	.751	.679	1.430	.289	1.926	.748	.853	1.601	.325
24.....	2.007	1.115	.708	1.823	.184	2.253	.888	.832	1.720	.533
25.....	2.183	1.250	.769	2.019	.164	2.451	1.364	.877	2.241	.210
26.....	2.237	.823	.802	1.625	.612	2.513	1.094	.843	1.937	.576
27.....	2.218	1.161	.767	1.928	.290	2.491	1.342	.881	2.223	.268
28.....	2.401	1.416	.713	2.129	.272	2.697	1.581	.912	2.493	.204
29.....	2.375	1.442	.711	2.153	.222	2.663	1.584	.858	2.442	.221
30.....	2.253	1.455	.779	2.234	.019	2.526	1.558	.922	2.480	.046
31.....	2.352	1.405	.734	2.139	.213	2.714	1.630	.887	2.517	.197
32.....	2.469	1.469	.787	2.256	.213	2.676	1.606	.876	2.482	.194
33.....	2.681	1.545	.885	2.430	.251	2.727	1.575	.907	2.482	.245
34.....	2.725	1.682	.839	2.521	.204	2.661	1.660	.812	2.472	.189
35.....	.....	.....	.....	.....	.....	2.631	1.654	.968	2.622	.009
36.....	.....	.....	.....	.....	.....	2.642	1.607	.927	2.534	.108
37.....	.....	.....	.....	.....	.....	2.595	1.581	.942	2.523	.072

[a] Removed at end of thirty-fourth week.

TABLE V.—*Nitrogen metabolism of steers*—Continued

FULL FEED LOT

[Expressed as pounds per period of seven days]

Week No.	Steer 661.[a]					Steer 663.[b]				
	Nitrogen ingested.	Nitrogen excreted.			Balance.	Nitrogen ingested.	Nitrogen excreted.			Balance.
		In urine.	In feces.	In urine and feces.			In urine.	In feces.	In urine and feces.	
1	.....	.....	.....	.....	.....	2.557	0.491	1.469	1.960	0.597
2	.....	.....	.....	.....	.....	2.612	.530	1.449	1.979	.683
3	2.959	0.653	1.641	2.294	0.665	2.837	.573	1.509	2.082	.755
4	2.838	.580	1.632	2.212	.626	2.542	.631	1.571	2.202	.340
5	2.694	.700	1.852	2.552	.142	2.410	.687	1.459	2.146	.264
6	2.797	.667	1.738	2.405	.392	2.542	.705	1.427	2.132	.410
7	2.540	.753	1.664	2.417	.123	2.367	.745	1.319	2.064	.303
8	2.468	.724	1.579	2.303	.165	2.292	.703	1.293	1.996	.296
9	2.591	.724	1.474	2.198	.393	2.418	.746	1.233	1.979	.439
10	2.595	.838	1.327	2.165	.430	2.427	1.154	1.228	2.382	.045
11	2.590	.865	1.509	2.374	.216	1.947	.779	1.122	1.901	.046
12	2.637	.790	1.466	2.256	.381	1.964	.620	1.154	1.774	.190
13	2.549	.837	1.536	2.373	.176	2.115	.606	1.054	1.660	.455
14	2.531	.757	1.498	2.255	.276	2.140	.635	1.232	1.867	.273
15	2.133	.775	1.243	2.018	.115	1.954	.866	.882	1.748	.206
16	1.828	.641	1.024	1.665	.163	1.313	.665	.690	1.355	—.042
17	1.832	.589	.934	1.523	.309	1.621	.445	.842	1.287	.334
18	1.840	.587	1.043	1.630	.210	1.541	.516	.808	1.324	.217
19	2.031	.589	1.068	1.657	.374	1.650	.512	.803	1.315	.335
20	1.778	.748	.915	1.663	.115	1.785	.634	.798	1.432	.353
21	2.064	.659	.987	1.646	.418	1.787	.670	.802	1.472	.315
22	1.741	.719	.944	1.663	.078	1.686	.615	.700	1.435	.251
23	2.434	.725	1.029	1.754	.680	2.118	.886	.848	1.734	.384
24	2.847	1.357	1.051	2.408	.439	2.481	1.309	.905	2.214	.267
25	3.089	1.619	1.103	2.722	.367	2.396	1.409	.746	2.245	.151
26	3.167	1.189	1.091	2.280	.887	2.320	1.408	.695	2.103	.217
27	3.140	1.674	1.126	2.800	.340	2.683	1.672	.845	2.517	.166
28	3.308	1.754	1.159	2.913	.485	2.950	1.683	.772	2.455	.495
29	3.383	1.990	1.104	3.094	.289	2.573	1.815	.670	2.485	.088
30	3.209	1.951	1.133	3.084	.125	1.692	1.625	.551	2.176	—.484
31	3.474	1.886	1.122	3.008	.466	.....	.....	.....	.....	.....
32	3.435	1.873	1.160	3.033	.402	.....	.....	.....	.....	.....
33	3.491	2.069	1.049	3.118	.373	.....	.....	.....	.....	.....
34	3.406	2.056	1.027	3.083	.323	.....	.....	.....	.....	.....
35	3.368	2.023	1.068	3.091	.277	.....	.....	.....	.....	.....
36	3.381	1.920	1.179	3.099	.282	.....	.....	.....	.....	.....
37	3.158	1.930	1.037	2.967	.191	.....	.....	.....	.....	.....

[a] Data obtained for first and second weeks.
[b] Removed at end of thirtieth week.

From the curve showing the live weights of the two steers it is seen that they made considerable gain during the experiment. However, from the eighth to the twenty-second week they made practically no gain. During this time the steers consumed from 0.40 to 0.45 pounds of digestible crude protein daily per 1,000 pounds live weight. In this connection it should be noted that 0.60 pounds of digestible crude protein

is generally accepted as the minimum for maintenance of cattle. The curves for the nitrogen balance show that the amount of protein consumed during this time was not only sufficient for maintenance but that there was a considerable storage of nitrogen. During this time—the eighth to the twenty-second week—the net energy consumption was a little higher than the usually accepted standard. The steers consumed from 6.7 to 7 therms, while 6 therms are considered the requirement for maintenance. In this connection it may be noted that from the thirty-fourth to the thirty-seventh week steer 656, when receiving 6 therms of energy, made a daily gain of ¾ pound per day.

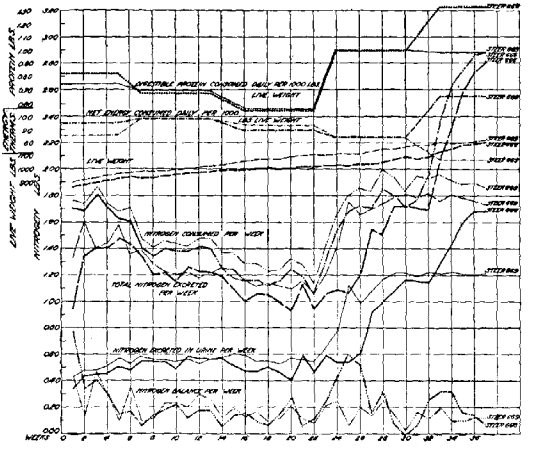

FIG. 2.—Nitrogen metabolism of steers in the one-third feed lot.

Note how nearly parallel are the curves showing the nitrogen consumption, the total nitrogen excretion, and the urinary nitrogen. The curve showing the nitrogen balance, while following the general trend of the nitrogen consumption, is much more irregular. Usually a slight decrease in the amount of nitrogen consumed caused a greater decrease in the amount of nitrogen stored, while a considerable increase in the amount of nitrogen consumed resulted in a much smaller increase in the amount of nitrogen stored.

ONE-THIRD FEED LOT.—Figure 2 shows the same data for the two steers of the one-third feed lot. It will be noted that in the same periods the consumption of digestible protein and net energy was considerably greater for the one-third feed steers than for the maintenance steers. Consequently the increase in live weight was more rapid and more un-

form. Apparently, however, there is but little relation between the curves showing the live weights and the curves showing the protein and energy consumption.

As with the maintenance lot, the curves showing the nitrogen consumption, the nitrogen excretion, and the urinary nitrogen are more or less parallel from week to week. Though in general the curves showing the nitrogen balance tend to follow the nitrogen consumption, yet there are many instances where they do not. As might be expected, the storage of nitrogen was greater in the one-third feed lot than in the maintenance lot. It also may be noted that in the one-third feed lot the storage of nitrogen was more irregular than in the maintenance lot.

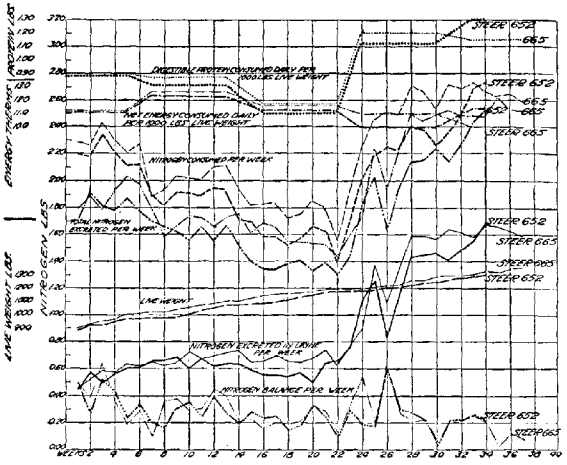

FIG. 3.—Nitrogen metabolism of steers in the two-thirds feed lot.

TWO-THIRDS FEED LOT.—Figure 3 gives the data for the steers of the two-thirds feed lot. It is seen that the consumption of protein and energy was still further increased, resulting in greater gains as shown by the more rapid incline of the live-weight curve.

The curves showing the total nitrogen excretion and the urinary nitrogen again follow the curve of the nitrogen consumption quite closely. The storage of nitrogen again follows the nitrogen consumption more or less closely with numerous irregularities. In general the curve is higher than in either of the lots previously studied.

FULL FEED LOT.—Figure 4 gives the corresponding curves for the full feed lot. It is noted that the consumption of protein and energy was somewhat greater than in the preceding lot, resulting in still better gains

in live weight. Steer 663 of this lot went off feed and had to be removed from the experiment at the end of the thirtieth week.

The relation between the nitrogen consumption and nitrogen balance was about the same as in the preceding lots, with more numerous and more striking irregularities. The nitrogen balance was generally greater than in the two-thirds feed lot, although there were many weeks when the reverse was true.

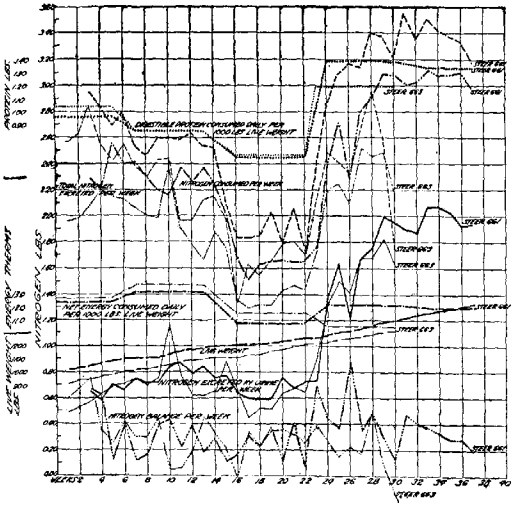

FIG. 4.—Nitrogen metabolism of steers in the full feed lot.

PERCENTAGE OF PROTEIN IN GAIN

In an experiment covering the length of time of this one it is reasonably accurate to calculate the percentage of protein in the total gain in live weight from the amount of nitrogen stored and the increase in live weight. This is particularly true when one considers the entire experiment of 37 weeks. The results are given in Table VI.

TABLE VI.—*Percentage of protein in gain*

[Results expressed in percentages]

Period No.	Weeks included in experiment.	Ratio of hay to corn to oil-meal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.		Average of all steers.
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.	Steer 665.	Steer 663.[a]	Steer 661.	
1	1–5	1:1:0	11. 02	10. 14	13. 99	14. 42	11. 50	10. 92	13. 87	16. 79	12. 83
2	8–13	1:3:0	(b)	(b)	11. 50	15. 23	11. 30	20. 27	10. 21	13. 41	13. 65
3	17–22	1:5:0	(b)	(b)	21. 20	9. 26	11. 90	18. 75	12. 71	24. 68	13. 08
4	25–30	1:4:1	15. 17	9. 68	18. 53	8. 33	13. 53	12. 10	5. 18	14. 28	12. 10
5[c]	34–37	1:4:1	10. 43	22. 73	7. 31	11. 50	[d]11. 36	6. 09		11. 95	11. 62
Total	1–37		14. 95	21. 15	14. 55	13. 15	13. 52	13. 32	11. 68	15. 37	14. 71

[a] Steer 663 removed at end of thirtieth week.
[b] Amount of protein stored greater than gain in live weight.
[c] Steers 650, 666, and 652 were on full feed in period 5.
[d] Steer 652 removed at end of thirty-fourth week.

From this table it is seen that there is no indication that the steers getting the larger amounts of nitrogen and energy showed any larger proportion of protein in their increase in live weight. Neither is there any indication that there was any difference in the percentage of protein in the increase in live weight during any period because of differences in the ration or differences in the age of the steers. Considering the average of all steers for the entire 37 weeks of the experiment, we find that 14.71 per cent of the total increase in live weight was protein (nitrogen × 6.25).

Jordan,[1] in an experiment with steers between the ages of 23 and 33 months, found by means of comparative slaughter tests that the increase in live weight during this time contained 13.57 per cent of protein. Waters, Mumford, and Trowbridge,[2] at the Missouri Experiment Station, found by means of comparative slaughter tests upon steers of similar age and size that the first 500 pounds of gain—that is, the increase in live weight from 748 to 1,248 pounds—contained 11.9 per cent of protein. Thus it is seen that while the method of experimentation used by Jordan and by Waters was entirely different from our own, yet quite similar results were obtained.

PERCENTAGE OF DIGESTED PROTEIN RETAINED

Table VII shows the percentage of the digested protein which was retained in the body by the individual steers. With the exception of the maintenance steers in period 1, there is no indication that the steers receiving larger amounts of digestible protein and net energy stored any more of the protein digested. In period 2 all steers stored less protein than they did in period 1, although there is no distinctive difference between lots. This decrease (from an average of 41.64 per cent

[1] Recalculated by Armsby. (ARMSBY, H. P. NUTRITION OF FARM ANIMALS. p. 372. New York, 1917.)
[2] BULL, Sleeter. PRINCIPLES OF FEEDING FARM ANIMALS. p. 32. New York, 1916.

to 27.41 per cent) may have been due to the smaller amounts of protein received in this period, or to the increase in the age of the steers, or to both. In period 3, when both the protein and energy were decreased, some of the steers stored more and others less protein. However, in period 4, when the protein was practically doubled and the energy slightly decreased, there was a considerable decrease in the percentage of protein stored, except for one steer, No. 666. The results obtained in period 5 are so irregular as to be of little value, although with one exception, steer 650, which was on full feed in this period, the results are usually lower. In general, our results indicate that a smaller percentage of the protein is retained as the age of the animal increases. As already pointed out, however, the protein and energy consumption for different periods varied, and this variation probably materially affects the value of our results.

TABLE VII.—*Percentage of digested protein retained*

Period No.	Weeks included in experiment.	Ratio of hay to corn to oilmeal.	Maintenance lot.		One-third feed lot.		Two-thirds feed lot.		Full feed lot.		Average of all steers.
			Steer 650.	Steer 656.	Steer 666.	Steer 669.	Steer 652.	Steer 665.	Steer 663.[a]	Steer 661.	
1.....	1–5	1:1:0	36.55	36.46	47.73	36.76	42.41	42.85	47.94	42.37	41.63
2.....	8–13	1:3:0	25.25	25.04	23.94	28.80	30.66	34.52	24.20	26.91	27.41
3.....	17–22	1:5:0	25.60	28.56	21.40	19.44	26.07	24.41	34.62	27.83	25.99
4.....	25–30	1:4:1	16.65	9.60	24.23	11.00	17.32	15.23	6.10	19.65	14.97
5[c b].....	34–37	1:4:1	27.87	[c]0.25	9.76	8.72	[d]10.58	5.54	.....	11.90	12.39
Total..	1–37	.....	25.06	18.92	22.93	18.98	23.98	21.70	24.03	23.33	22.37

[a] Steer 663 removed at end of thirtieth week.
[b] Steers 650, 666, and 652 were on full feed in period 5.
[c] Not included in average of all steers.
[d] Steer 652 removed at end of thirty-fourth week.

A study of the results for all steers for the entire 37 weeks of the experiment shows no distinctive differences between lots. In fact individual differences are quite small, considering the nature of the experiment. The results show that the eight steers stored from 18.92 to 25.06 per cent, or an average of 22.37 per cent, of the protein digested.

SUMMARY

(1) The results pertaining to the nitrogen metabolism of eight 2-year-old steers for a period of 37 weeks are given.

(2) Steers maintained a positive nitrogen balance for long periods of time when receiving considerably smaller amounts of digestible protein than are usually considered necessary for maintenance.

(3) Curves showing the nitrogen consumption, the total nitrogen excretion, the urinary nitrogen, and the nitrogen balance are more or less parallel.

(4) Steers receiving larger amounts of nitrogen stored larger amounts.

(5) The amount of nitrogen consumed had no effect upon the percentage of protein in the increase in live weight.

(6) Differences in the ration and in the age of the steers had no effect upon the percentage of protein in the increase of live weight.

(7) An average of the results for eight steers for 37 weeks shows that 14.71 per cent of the increase in live weight was protein.

(8) The amount of protein and energy consumed had no effect upon the percentage of the protein retained.

(9) It is indicated that a smaller percentage of the protein is retained as the age of the animal increases.

(10) An average of the eight steers for 37 weeks shows that 22.37 per cent of the protein digested was stored in the body.

DEVELOPMENT OF THE PISTILLATE SPIKELET AND FERTILIZATION IN ZEA MAYS L.[1]

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INTRODUCTION

During the past five years considerable time has been given to a cytological study of the pistillate spikelet and flower of the corn plant (*Zea mays*). This work was undertaken with the primary idea of obtaining some facts that could be used in the advanced instruction of students in agriculture, since the cytological work that has been reported for the more common crop plants is limited and fragementary. The lack of investigations of this kind has long been felt not only by those giving instruction to students in botany, agronomy, and plant breeding but also by those who are concerned with investigations in the practical breeding and improvement of crop plants.

REVIEW OF LITERATURE

Crozier (*2*)[2] found that the silk of corn would remain in a receptive condition and grow in length for a long time if pollination was prevented. He also found that it was not alone the forked tip of the silk that was receptive to pollen but that fertilization could be effected by the pollination of the silks after the branched tips had been removed. True (*14*) studied the development of corn, wheat, and oats from the time of fertilization to the maturity of the seed. He described the pistillate flower of corn only in so far as it would be of aid to him in discussing the formation of the caryopsis. Guignard (*4*) described in considerable detail the structure of the ovary and ovule of corn and observed the process of double fertilization but published no drawings of his observations. Poindexter (*11*) described the development of the pistillate spikelet of corn and discussed briefly the early stages in the development of the

[1] Published with the approval of the Director. Contribution from the Department of Botany, Kansas Agricultural Experiment Station, paper No. 31.

[2] Reference is made by number (italic) to "Literature cited," p. 264–265.

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embryo and endosperm. Kuwada (*9*) made a cytological study of the pollen mother cells of a number of varieties of corn. He found that there was a considerable variation in the size and number of the chromosomes even in the same race. The haploid number varied from 9 to 12, the higher number as a rule being found in the varieties of sugar corn and the lower numbers in the varieties with more starch. In a later paper (*10*) he reported that the diploid number of chromosomes varied from 20 to 22 in the cells of the root, but that the number was constant for any one plant. Weatherwax (*15–17*) since this work has been in progress has reported extensively on the development, structure, and evolution of both the pistillate and staminate spikelets of the corn plant. Further mention of his work will be made in the discussion of the results reported in the present article.

EXPERIMENTAL METHODS

The varieties of corn used in this work were Pride of Saline, Freed White Dent, and Sherrod White Dent. The material was collected in the field at Garden City, Kans., during the seasons of 1914 to 1917, and at Manhattan, Kans., in 1918. All the material used in this investigation was fixed in medium chrom-acetic solution, washed, dehydrated, cleared in xylol, and embedded in paraffin in the usual manner. The sections for the most part were cut 15 to 20 microns in thickness and stained with safranin, gentian violet, and orange G. The drawings of the developing spikelet were made with the aid of a Bausch and Lomb projection apparatus, and those showing the development of the embryo sac and fertilization were made by the aid of the camera lucida.

In order to study the time elapsing between pollination and fertilization, the young ears were bagged before the silks appeared. After the silks had practically all appeared, they were hand-pollinated with freshly collected pollen. After pollination the ears were again bagged and kept covered until the specimens were collected for fixing. The ears which furnished the material for study were collected at stated hourly intervals after pollination had been made. In this manner the time elapsing between the time of pollination and fertilization could be determined.

For the study of the course of the pollen tube, the silks at certain periods after pollination were cut into short lengths and then tied into small bundles by means of fine threads. These bundles were then fixed and embedded in the same way as the other material. By cutting the bundles lengthwise, a large number of silks for a portion of their length could be obtained in longitudinal section. Since the bundles were taken consecutively from the tip of the silk to the ovary, the course of the pollen tube could be observed in any portion of the silk.

EXPERIMENTAL DATA

MATURE PISTILLATE SPIKELET

The pistillate spikelet of corn at the time the silk emerges from the husk has the appearance in longitudinal section shown in Plate 19. The two empty glumes of the spikelet are thickened at their base but are thin and membranous at their tips. The spikelet bears two flowers, but in most cases one of these aborts, so that in each spikelet there is only one functional flower. Each of the flowers of the spikelet consists of a pistil and three stamens. The stamens in both flowers, however, remain rudimentary, so that the only part of the fertile flower that functions is the pistil. The development and disorganization of the stamens as well as the development and abortion of the pistil of the sterile flower have been described in much detail by Weatherwax (*16*). Each flower is subtended by a lemma or flowering glume. Between the two flowers and adjacent to each other are located the two palets. The palets and lemmas are much shorter and more membranous than the empty glumes. With the exception of the pod corns, the bracts of the spikelet cease growth at the time of fertilization and thus never completely inclose the ovary. The bracts remain at the base of the grain and form the chaff of the cob. If fertilization is not effected, however, the bracts of the spikelet continue to grow in length and will completely inclose the ovary of the fertile flower. In pod corn, the bracts continue to grow after fertilization and completely inclose the mature grain.

When the spikelet is mature the lodicules of the fertile flower are not present or are not easily seen. According to Weatherwax (*16*) the lodicules in early stages of growth are present in both flowers, but those of the functional flower are crowded out before it is mature, while those of the sterile flower remain intact and can readily be observed even when that flower has a functional pistil.

The pistil of the fertile flower consists of the ovary and the elongated style or "silk." The silk is unevenly cleft at its tip, and this branched portion has been termed the stigma of the pistil by most authors. A small rounded knob or protuberance is located at the top of the ovary near the base of the silk. In the center of this knob is a funnel-shaped depression, apparently leading to the cavity of the ovary. However, an examination of a section through this region shows that the depression is only superficial and that the opening which at one time led to the cavity of the ovary has been closed. The cells composing the wall of this cavity have never completely united (Pl. 19, sc). This incomplete union of the wall of the ovary was noticed by True (*14*) but was first termed the stylar canal by Guignard (*4*). The origin of this canal will be discussed in detail when the embryonic development of the spikelet is considered.

The ovule is of a modified campylotropous type and is attached by approximately one-third of its circumference to the bottom of the cavity of the ovary. The outer coat of the ovule is incomplete and extends about half way around it. The outer coat for its whole length, with the exception of a short distance at the base, is free from the inner coat. The inner coat fits closely to the ovule and covers it completely, except in the region of the micropyle. Each coat of the ovule is approximately two cells in thickness, except in the region of the micropyle and the stylar canal where the coats may be from three to four cells in thickness. The outer coat forms a wedge-shaped projection which extends into the inner depression of the stylar canal. The inner coat also often shows such a projection, but it is never so marked as that in the outer coat (Pl. 19, ovc). This projection of the outer coat into the stylar canal has been observed by both Guignard (*4*) and Weatherwax (*16*).

The two fibro-vascular bundles of the silk traverse the walls of the ovary and unite at its base with the bundles that supply the various elements of the spikelet. Extending from each of the fibro-vascular bundles of the silk to the cavity of the ovary is a bundle of elongated cells that are rich in protoplasm and resemble very closely the sheath cells of the fibro-vascular bundles of the silk. Through these sheathlike cells the pollen tube travels to the cavity of the ovary after it leaves the sheath cells of the fibro-vascular bundles of the silk (Pl. 19, vbs, bsc).

DEVELOPMENT OF THE PISTILLATE SPIKELET

The spikelets are borne on the cob in double rows, because the spikelets are paired; and since each spikelet has only one functional flower, an even number of rows of grains is produced. It has been observed by Kempton (*7*), Stewart (*12*), and Weatherwax (*15*) that frequently in certain varieties both flowers of the spikelet may function, and thus two grains may be produced to each spikelet instead of one. In these varieties the grains do not always occur in regular rows on the cob but may be more or less irregularly arranged. This is due to the fact that the development of two grains in a spikelet tends to crowd the other grains in that region more or less out of alignment.

The origin of the paired spikelets is best observed by a study of the cross section of a very young cob. Such a cross section of the tip shows that it is composed of undifferentiated or embryonic cells (Pl. 20, A). A short distance back of the tip numerous projections or protuberances appear on the periphery of the cob. Each of these projections is a rudiment or primordium from which a pair of spikelets will develop (Pl. 20, B). Soon after the formation of these rudiments, each one becomes equally divided (Pl. 20, C), and from each half a spikelet develops (Pl. 20, D). The progressive development of a spikelet from its primordium is best studied in longitudinal section. The appearance of the embryonic cells

of the tip of a young cob in longitudinal section (Pl. 21, A) differs a little from that in cross section (Pl. 20, A).

A longitudinal section of the rudiment of a spikelet just after its appearance shows that it is composed of embryonic cells and has the general appearance of the tip of the young cob. The first differentiation to appear on the rudiment of the spikelet is the lower empty glume (Pl. 21, B). The primordium of the upper empty glume soon appears (Pl. 21, C), so that at a little later stage the two developing glumes have practically the same appearance (Pl. 21, D). The primordia of the two lemmas or flowering glumes are the next to appear (Pl. 22, A), while directly following, or frequently at the same time, the rudiments of the sterile flower and of the stamens of the fertile flower become visible. The palet of the fertile flower at this time also begins to show differentiation (Pl. 22, B), but the palet of the sterile flower does not appear until considerably later.

The primordium of the carpellate leaf or ovary wall of the fertile pistil begins to show in a short time after those of the palet and stamens of the fertile flower appear (Pl. 23, A). At this time the cells that are to compose the fibro-vascular bundles of the lower part of the spikelet begin to differentiate. The carpel grows unevenly so that when the side adjacent to the lemma extends almost one-third around the young ovule the opposite side has scarcely begun to develop (Pl. 23, A, c, c'). This more rapidly growing portion of the carpel increases in width toward the tip so that it becomes from two to three times wider than the base (Pl. 23, B). This widened portion of the carpel is composed of numerous embryonic cells which later rapidly elongate to form the silk (Pl. 24, A). When the silk is elongating, the wall of the ovary has grown up around the ovule and has almost inclosed it with the exception of a small opening toward the top (Pl. 24, A, sc). This opening has been termed the stylar canal. It, however, does not long remain open, for by the time the silk is ready for pollination, the edges of the carpel have come in close contact but have not grown together (Pl. 25, B).

About the time the silk begins to elongate, the ovule begins to invert. The cells of the ovule on the side adjacent to the palet increase in number and elongate more rapidly than those on the opposite side, thus causing the end of the ovule to turn downward (Pl. 24, B). The megaspore mother cell appears about the time the ovule begins to turn, and frequently the embryo sac has reached the 2-celled stage by the time the ovule has become completely inverted. The ovule coats grow rapidly when the ovule begins to curve, so that by the time it has reached its final position they have reached their full development (Pl. 25, A).

DEVELOPMENT OF THE EMBRYO SAC

About the time the ovule begins to invert, the differentiation of the megaspore mother cell becomes apparent (Pl. 24). No disorganization of any of the megaspores was noted in the three varieties of corn studied

in this experiment, although approximately 50 observations were made. This fact was also observed by Weatherwax (*16*) in the varieties of corn studied by him, so it seems to be the rule that all four megaspores function. In wheat (*Triticum vulgare*), however, Koernicke (*8*) and Jensen (*6*) report that only one megaspore functions. The same has been observed by Cannon (*1*) for wild oats (*Avena fatua*).

The megaspore mother cell increases in size until it becomes about twice as broad and from four to five times as long as the vegetative cells of the ovule (Pl. 26, A, B). The developing embryo sac remains approximately the same size as the megaspore mother cell until the eight cells are formed. At that time it has elongated but slightly while its breadth has increased to two or three times that of the megaspore mother cell (Pl. 27, B). The two polar nuclei migrate and come in contact with each other a short distance above the egg but do not fuse before fertilization takes place (Pl. 27, B, C). In scores of cases where pollination had been prevented the two polar nuclei were observed standing apart a week or more after the embryo sacs were ready for fertilization.

MATURE EMBRYO SAC

When the embryo sac is mature, it is approximately four times as long and about twice as wide as when it first reaches the 8-celled stage. It reaches its maximum size about the time the silk emerges from the husk. The antipodals begin to divide almost immediately after the 8 cells are formed, so that one very rarely finds an embryo sac that shows only 8 cells. The antipodals increase in number, apparently by indirect cell division, until they number from 24 to 36 cells at the time of anthesis. These cells often have indistinct walls, and frequently there are two nuclei to each cell. These cells are closely crowded together and give the appearance of a rather definite tissue (Pl. 28, ant). This behavior of the antipodals is characteristic of the grasses and has been noted by numerous investigators since the time of Hofmeister (*5*). Golinski (*3*) in his work with the stamens and pistil of wheat studied the antipodals with especial care in order to determine whether they played any part in the formation of the endosperm and established the fact that these cells remain intact until they are crowded out by the growing endosperm (Pl. 32, B).

The egg increases in size until its width is almost half that of the embryo sac. It is decidedly balloon-shaped and becomes areolar in appearance. The synergids are more or less lunar-shaped and are considerably longer than the egg. They have dense cell contents and take the stain much deeper than the egg. (Pl. 28, e, sy). The nuclei of the synergids may disintegrate before fertilization or may remain clear and distinct until it has taken place. In most cases the synergids do not remain long intact after the egg is ready for fertilization. Where

fertilization is delayed they lose their identity and can not be distinguished from the surrounding cytoplasm.

The polar nuclei are embedded in a strand of cytoplasm that extends from the antipodals to the egg, while the greater part of that portion of the embryo sac is taken up by two large vacuoles. The nucleoli of the polar nuclei are the largest in the embryo sac. The two polar nuclei remain in close contact but do not fuse until fertilization has taken place.

SILK AND THE POLLEN TUBE

The end of the silk is cleft into two branches of unequal length. This branched portion of the silk has been termed the stigma by most authors in their description of the corn flower (Pl. 29, A). The silk, however, is receptive to pollen for at least the greater portion of its length; so it would appear that Weatherwax (*16*) is correct in asserting that the term stigma can be applied to the branched tip of the silk only in a morphological sense and not with the understanding that it is the only portion of the pistil on which the pollen grains may germinate.

Numerous hairs are borne on the silk in rather definite areas for its entire length (Pl. 29, A). These hairs appear for the most part on the edges of the silk and are more numerous near its tip than farther down. The hairs may be branched or unbranched and the upper ends of the cells that compose them stand out from the hair (Pl. 29, B), thus forming a rough surface upon which the pollen grains easily lodge. The origin and development of these hairs have been described in detail by Weatherwax (*15*), who observed that each hair originates from a single epidermal cell of the silk.

Two fibro-vascular bundles extend the entire length of the silk and terminate in the branched tip (Pl. 29, A). A cross section of the silk shows that it is grooved on both its upper and lower surfaces and that the vascular bundles are located near its edge (Pl. 30, A). Each bundle contains from three to six xylem elements (Pl. 30, B). The conducting tissue of the fibro-vascular bundles is surrounded by narrow, elongated cells that are characterized by very dense cytoplasmic contents and elongated flattened nuclei (Pl. 30, C). It is between these dense cells that the pollen tube travels down the silk.

The pollen grains vary in shape from spherical to ellipsoidal, and each grain has a germ pore (Pl. 29, C). The protoplasm of the pollen grain is very dense, and often it is difficult to distinguish the nuclei. The two sperm nuclei are formed before the pollen is shed (Pl. 32, A). This supports the statement of Strasburger (*13*) that the division of the generative nucleus in the pollen grain is a constant character for all the grasses.

A few hours after the pollen grains lodge on the hairs of the silk, the pollen tube emerges from the germ pore (Pl. 29, D). Three ways have

been observed by which the pollen tube may gain access to the sheath cells of the fibro-vascular bundles of the silk. Shortly after the pollen tube appears, it may penetrate a hair and through it gain entrance to the fibro-vascular bundle region (Pl. 29, D); or the tube may continue down the outside of hair to its base and then enter the silk and penetrate to the cells surrounding the bundle. Frequently pollen grains that fall directly on the smooth portion of the silk germinate, and the pollen tube penetrates the silk. These instances, however, are exceptions. Practically all pollen tubes that function are from pollen grains that fall on the hairs of the silk.

The end of the pollen tube is greatly enlarged as it pushes its way between the dense sheath cells of the bundle (Pl. 29, E). In its passage down the silk the tube causes but very little disturbance in the position of the cells, so that after the tube disappears the cells quickly return to their normal form and position. The pollen tube, so far as I have observed, does not extend the full length of the silk at any time. It is very difficult to locate it a short distance back of its growing region. It appears that the older portions of the tube are absorbed by the surrounding cells, while the growing part of the tube apparently is nourished by the dense sheath cells. Arriving at the base of the silk, the pollen tube pushes its way between the sheathlike cells that extend from the bundle of the silk to the cavity of the ovary (Pl. 19, vsc). After it enters the ovary cavity the tube twists and coils in its passage along the coats of the ovule until it reaches the micropyle. After passing through the micropyle, the tube works its way between the cells of the ovule and enters the embryo sac (Pl. 28, pt). The protoplasm of the pollen tube is very dense, so that it is very difficult to locate the sperm nuclei. I have never observed them in the tube except after it had entered the embryo sac.

If pollen is supplied abundantly, a great number of pollen tubes start to grow down the bundle regions of each silk. However, as one examines the silk from the tip downward, the number of pollen tubes becomes smaller and smaller, so that when the cavity of the ovary is reached only one pollen tube is to be observed. In nearly a hundred observations no more than one pollen tube was seen in each ovary cavity.

The growth of the pollen tubes is very rapid, and under ordinary conditions they reach the embryo sacs of all the ovules on the ear in 24 hours after pollination. In order to do this the longest tubes must grow in that time approximately 6 inches, a distance that equals 1,500 times the diameter of the pollen grain.

FERTILIZATION

After the entrance of the pollen tube into the embryo sac, it expands so that the width of its tip is approximately one-third that of the embryo sac. The pollen tube extends into the embryo sac until the tip is near the polar nuclei. The wall of the tube is dissolved, giving the nuclei free access to the embryo sac. One of the sperm nuclei fuses with the egg and another with one of the polar nuclei (Pl. 31). The two polar nuclei fuse at the time the sperm nucleus enters one of them or shortly afterwards. Traces of the pollen tube in the embryo sac remain for a long time and do not disappear until crowded out by the developing endosperm and embryo. Fertilization takes place in from 26 to 28 hours after pollination, or in a few hours after the pollen tube reaches the embryo sac.

DEVELOPMENT OF THE EMBRYO AND ENDOSPERM

Almost immediately after fertilization, the endosperm nucleus begins to divide; and in 10 to 12 hours the nuclei of the endosperm may number 20 or 30, arranged around the periphery of the embryo sac (Pl. 32, A). Many of the nuclei have two nucleoli. The nucleus of the fertilized egg does not divide very rapidly. When the nuclei of the endosperm number as high as 20 or 30, the egg nucleus has just undergone its first division (Pl. 32, A). The cells of the endosperm increase very rapidly, and within 36 hours after fertilization they completely fill the embryo sac (Pl. 32, B). The antipodals remain intact and increase in number but are soon crowded out by the encroaching endosperm cells. By the time the endosperm completely fills the embryo sac the embryo consists of only from 14 to 16 cells (Pl. 32, C).

SUMMARY

In a study of the pistillate spikelet and the process of fertilization in the corn plant (*Zea mays*) the following facts were noted:

EMBRYO SAC.—In the formation of the embryo sac there is no disorganization of the megaspores, and all four function. The three antipodal cells rapidly increase in number, apparently by indirect cell division, until they number from 24 to 36 at the time the embryo sac is mature. These cells have rather indistinct cell walls and frequently contain two nuclei. The two polar nuclei come into position just above the egg and remain in close contact with each other but never fuse before fertilization has taken place. The egg becomes reticulate, stains very lightly, and is decidedly balloon-shaped.

POLLEN TUBE.—Practically all the pollen tubes that function come from the pollen grains that lodge on the hairs of the silk. The tubes may enter the hairs directly and through them gain access to the interior of the silk, or they may follow the hairs to their base and then penetrate the silk. After the pollen tubes are once inside the silk they work their

way between the cells to the fibro-vascular bundles. Each silk has two fibro-vascular bundles. These bundles are surrounded by sheath cells which are characterized by their extremely dense contents and large, flattened nuclei. It is between these cells that the pollen tube travels down the silk. Arriving at the base of the silk, the pollen tube works its way between the sheathlike cells that extend from the fibro-vascular bundle of the silk to the cavity of the ovary. The tube enters the ovary cavity and twists and coils in its passage along the ovule coat until it reaches the micropyle. The tube then pushes between the cells of the ovule until it reaches the embryo sac. The growth of the pollen tubes is very rapid, so that they reach the embryo sacs of all the ovules of the ear in 24 hours after pollination. To do this some of the tubes must grow a distance of approximately 6 inches in the course of the 24 hours. The pollen tubes apparently do not extend the full length of the silk at any given time but are absorbed a short distance back of their tip by the cells between which they pass. A great number of tubes start down a given silk; but the number of tubes becomes less and less as the base of the silk is approached, so that by the time the cavity of the ovary is reached only one tube is to be observed. The two sperm nuclei are formed in the pollen grain before the pollen tube appears.

FERTILIZATION.—The pollen tube enters the embryo sac and pushes its way upward until its tip is near the polar nuclei. The tip of the tube expands until it is approximately one-third the width of the embryo sac. The wall of the tube seems to dissolve, giving the sperm nuclei access to the embryo sac. One of the sperm nuclei fuses with the egg, and at about the same time the other fuses with one of the polar nuclei. The two polar nuclei fuse at the time the sperm nucleus enters one of them or shortly afterwards. The pollen tube persists in the embryo sac until it is crowded out by the developing endosperm and embryo. Fertilization occurs in from 26 to 28 hours after the silks have been pollinated.

ENDOSPERM AND EMBRYO.—The endosperm nucleus soon divides, and in from 10 to 12 hours after fertilization the endosperm nuclei may number as high as 30, arranged around the periphery of the embryo sac. Within 36 hours after fertilization the cells of the endosperm completely fill the embryo sac. The nucleus of the ferilized egg does not divide for some time, so the endosperm may number 20 or more cells before the first division of the egg takes place. When the cells of the endosperm completely fill the embryo sac, the embryo numbers only 14 to 16 cells.

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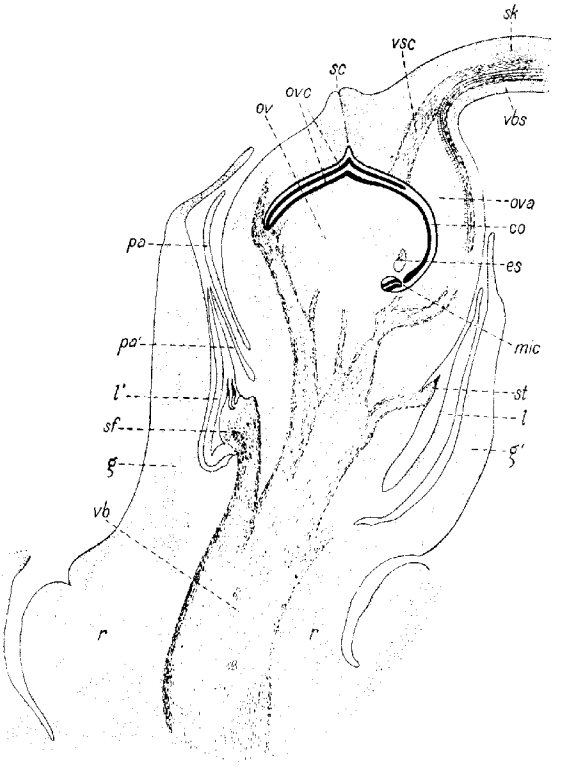
PLATE 19

Longitudinal section of the pistillate spikelet of corn at the time the silk is ready for pollination: r, rachilla; g, lower empty glume; g′, upper empty glume; l, lemma or flowering glume of the fertile flower; l′, lemma or flowering glume of the sterile flower; pa, palet of the fertile flower; pa′, palet of the sterile flower; sf, sterile flower; st, rudimentary stamen of the fertile flower; ova, ovary of the pistil; co, cavity of the ovary; sk, silk or style; sc, stylar canal; vbs, one of the fibro-vascular bundles of the silk.

Through the sheath cells that surround the bundle the pollen tube travels down the silk; vsc, sheathlike cells through which the pollen tube travels from the vascular bundle to the cavity of the ovary; vb, fibro-vascular bundles that supply the parts of the spikelet; ov, ovule; ovc, ovule coats; mic, micropyle; es, embryo sac. × 45.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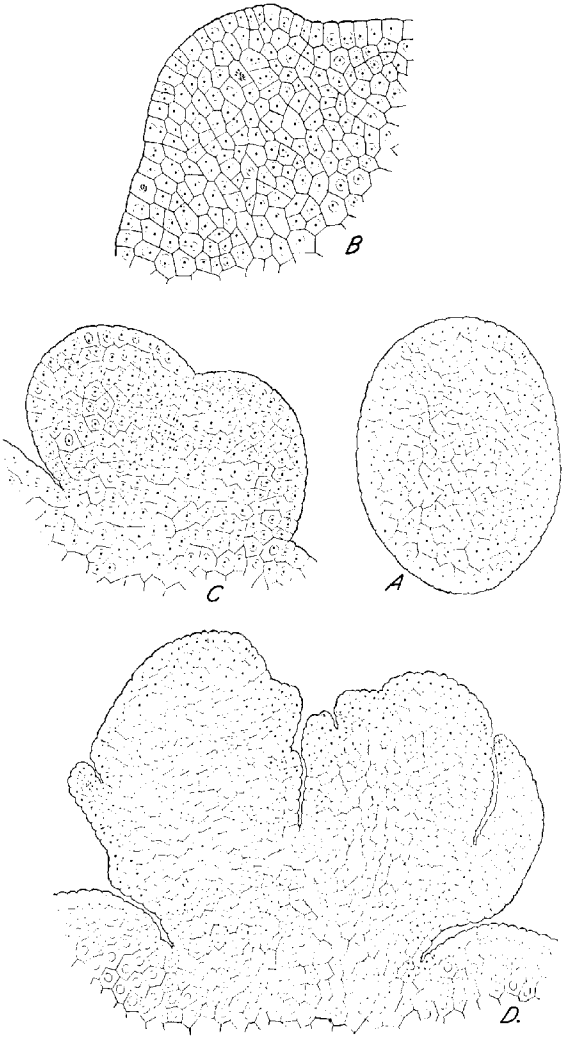

B
C
A
D.

PLATE 20

A.—Cross section of the tip of a very young cob. × 300.

B.—Portion of a cross section of a young cob just back of the tip, showing the rudiment or primordium from which a pair of spikelets will develop. × 300.

C.—Cross section of a rudiment at the beginning of its division into equal parts. × 300.

D.—Cross section of a pair of spikelets in the process of development. × 300.

PLATE 21

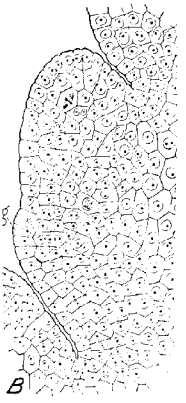
A.—Longitudinal section of the tip of a young cob. × 300.

B.—Longitudinal section of the rudiment or primordium of a spikelet just back of the tip of a young cob: g, primordium of the lower empty glume. × 300.

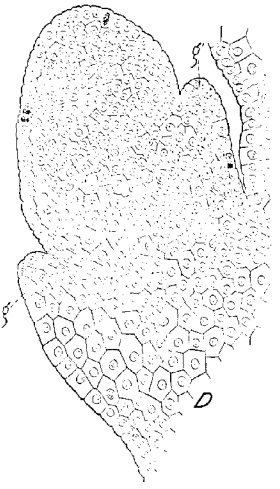
C.—Longitudinal section of the developing spikelet, showing the primordia of the lower and upper empty glumes: g, lower empty glume; g′, upper empty glume. × 300.

D.—Longitudinal section of the developing spikelet at a little later stage than C: g, lower empty glume; g′, upper empty glume. × 300.


A


B


C


D

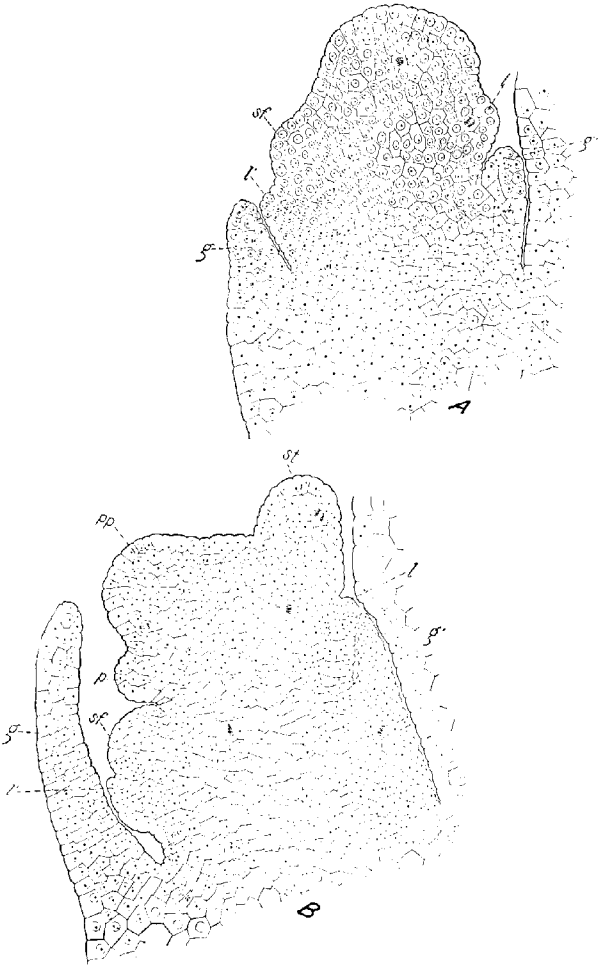

sf
l'
g'
A
sf
pp
p
l
g'
g''
l''
B

PLATE 22

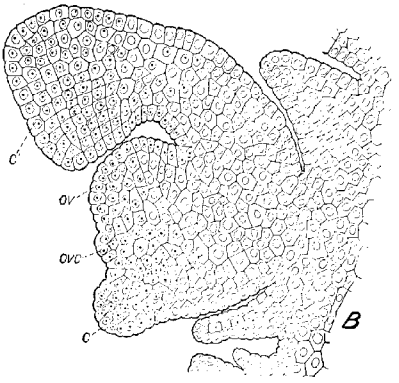
A.—Longitudinal section of the developing spikelet: g, lower empty glume; g′, upper empty glume; l, primordium of the lemma or flowering glume of the fertile flower; l′, primordium of the lemma or flowering glume of the sterile flower; sf, primordium of the sterile flower. × 300.

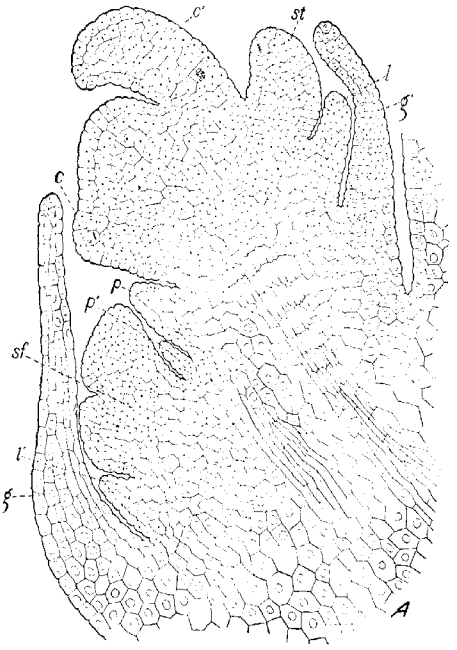
B.—Longitudinal section of the developing spikelet: g and g′, empty glumes; l and l′, lemmas or flowering glumes; sf, primordium of the sterile flower; st, stamen of the fertile flower; p, palet of the fertile flower; pp, primordium of the pistil. × 300.

PLATE 23

A.—Longitudinal section of the developing spikelet at the time the carpel or ovary wall has begun to develop: g and g′, empty glumes; l and l′, lemmas; sf, sterile flower; p and p′, palets; st, stamen; c and c′, rudiment of the carpel or ovary wall; c′ is the more rapidly growing part of the carpel. × 300.

B.—Longitudinal section of a developing ovary: c and c′, developing ovary walls; c′ is the portion of the carpel from which the style or silk will develop; ov, ovule; ovc, primordium of the inner ovule coat. × 300.


c'
ov'
ovc'
c'
B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st
l
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c
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p'
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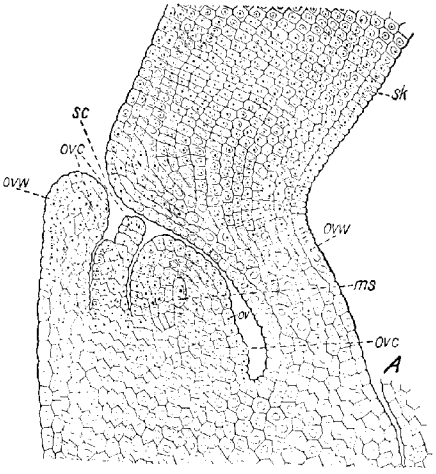
A


sk
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ov
ovc
A

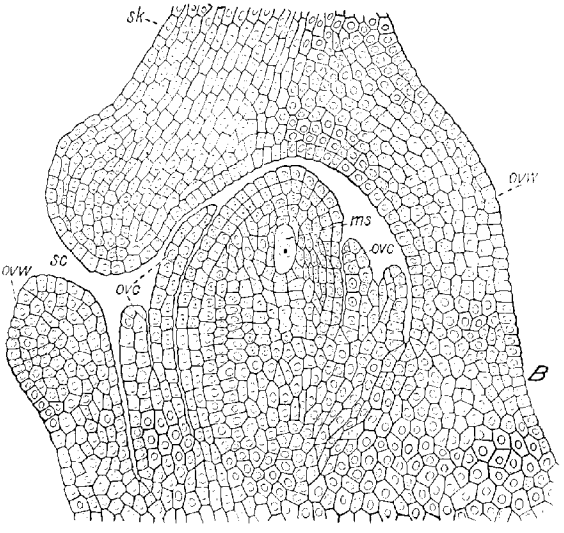

sk
ovw
ms
ovc
ovw
sc
ovc
B

PLATE 24

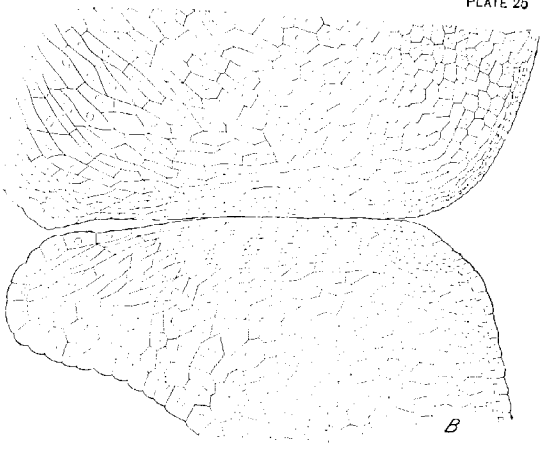
A.—Longitudinal section of the fertile pistil at the time the silk has started to elongate: ovw, ovary wall; sc, stylar canal; ovc, ovule coats; ov, ovule; ms, megaspore mother cell; sk, silk. × 300.

B.—Longitudinal section of the fertile pistil at the time the ovule has started to invert: ovw, ovary wall; sc, stylar canal; sk, silk; ovc, ovule coats; ms, megaspore-mother cell. × 300.

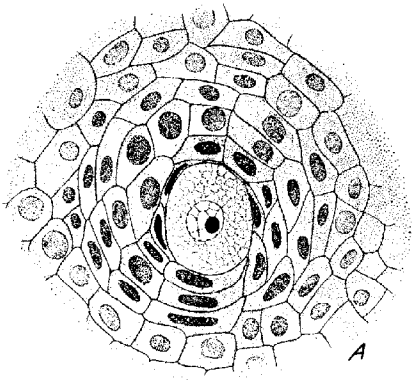
PLATE 25

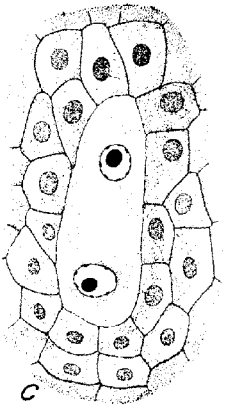
A.—Longitudinal section of the inverted ovule: ov, ovule; ovc, ovule coats; es, embryo sac; mic, micropyle; co, cavity of ovary. × 250.

B.—Section through the stylar canal, showing its structure shortly before the silk emerges from the husk. The union of the two edges of the carpel will eventually be more complete near the top than is here shown. × 250.


B


Ov C
CO
OV
ES


A


C

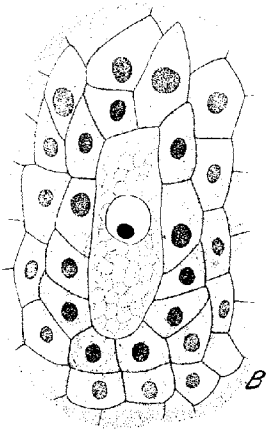

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PLATE 26

A.—Cross section of the megaspore mother cell. × 800.
B.—Longitudinal section of the megaspore mother cell. × 800.
C.—Longitudinal section of the 2-celled embryo sac. × 800.

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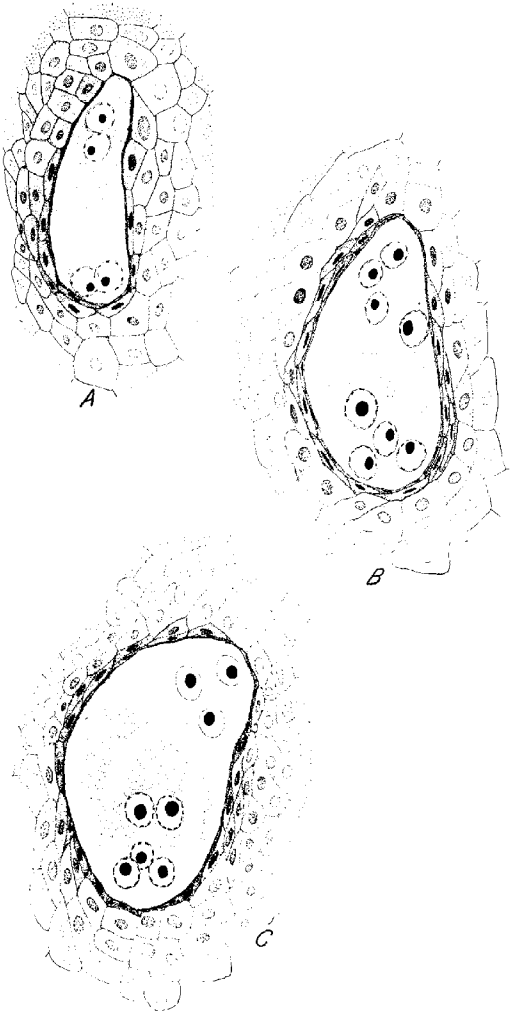
PLATE 27

A.—Longitudinal section of the 4-celled embryo sac. × 800.

B.—Longitudinal section of an 8-celled embryo sac at the time the polar nuclei have started to migrate. × 800.

C.—Longitudinal section of an 8-celled embryo sac after the polar nuclei have migrated. × 800.


A
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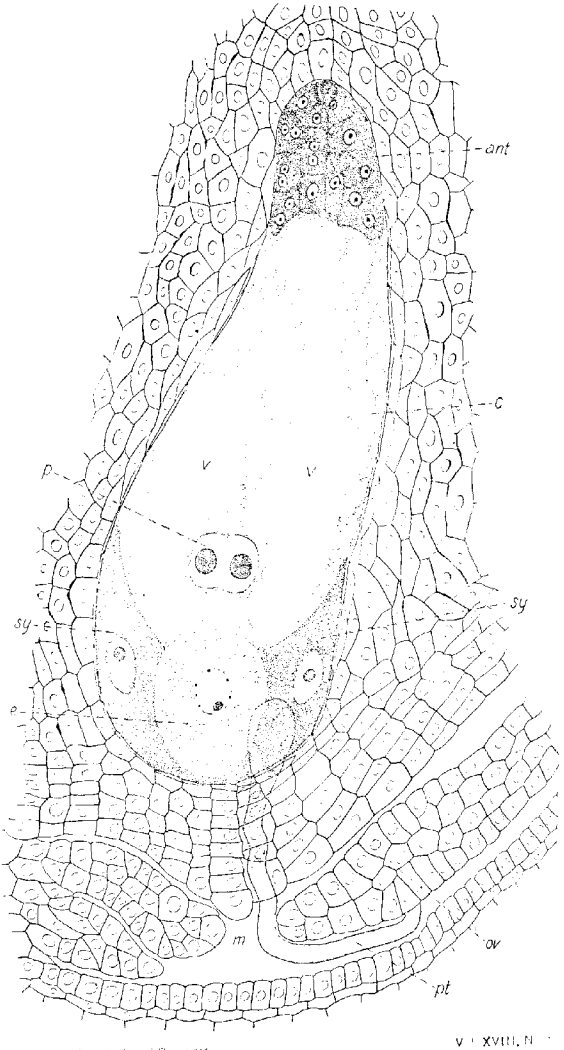
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sy
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p
m
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PLATE 28

Longitudinal section of a mature embryo sac just previous to fertilization: e, egg; sy, synergids; p, polars; ant, antipodals; pt, pollen tube; m, micropyle; v, vacuole; c, cytoplasm; ov, ovule coat. × 520.

PLATE 29

A.—End of a silk: p, pollen grains; v, fibro-vascular bundles; h, hairs. × 35.

B.—Tips of the hairs of the silk. It is on these hairs that most of the pollen grains lodge. × 250.

C.—Section of a pollen grain showing the germ pore and the relative size of the vegetative and sperm nuclei. × 250.

D.—Single hair of the silk, showing the general manner in which the pollen tube penetrates the sheath cells of the fibro-vascular bundle of the silk: p, pollen grain; h, hair; pt, pollen tube; sc, sheath cells of the fibro-vascular bundle. × 250.

E.—Longitudinal section of a fibro-vascular bundle of a silk, showing the position of the pollen tube as it grows down the silk: sc, sheath cells; x, xylem elements; p, parenchyma cells of the silk; pt, pollen tube, showing the enlarged tip. × 250.

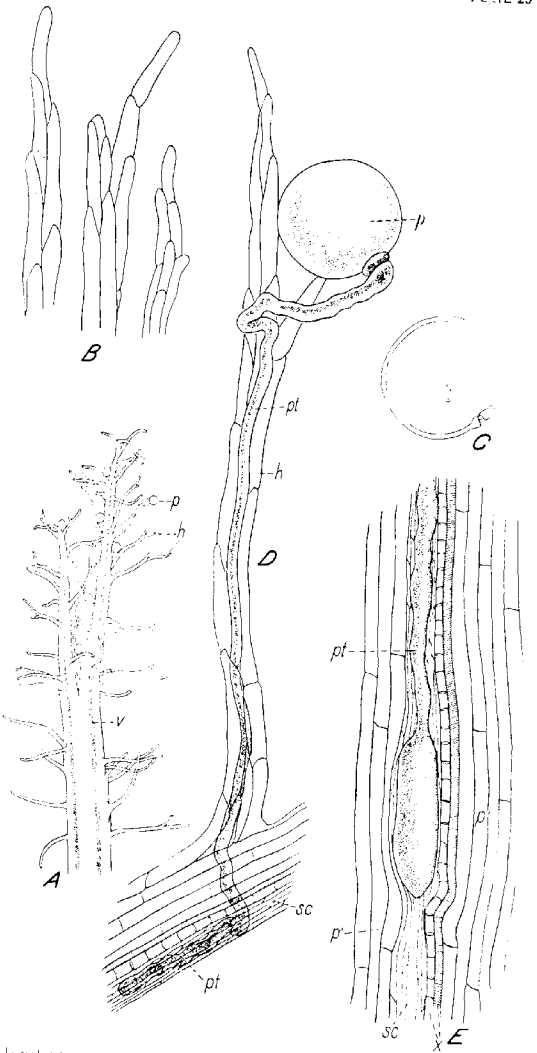
B
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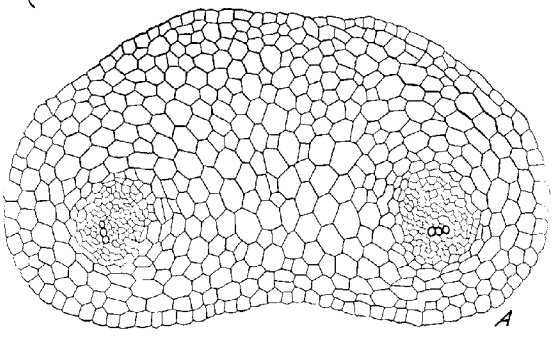
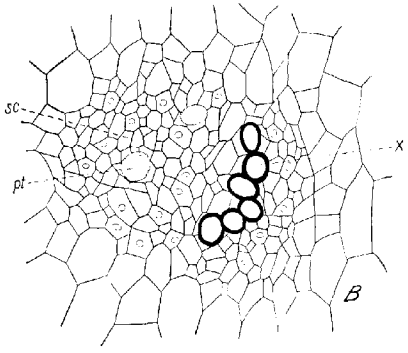
Plate Spikelet and Fertilization in Zea mays L.





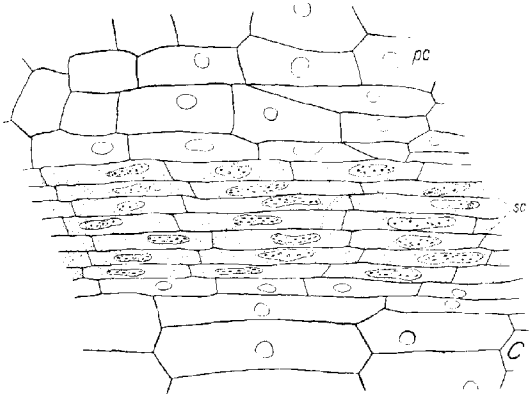


PLATE 30

A.—Cross section of a silk near its base, showing the position of the fibro-vascular bundles. × 220.

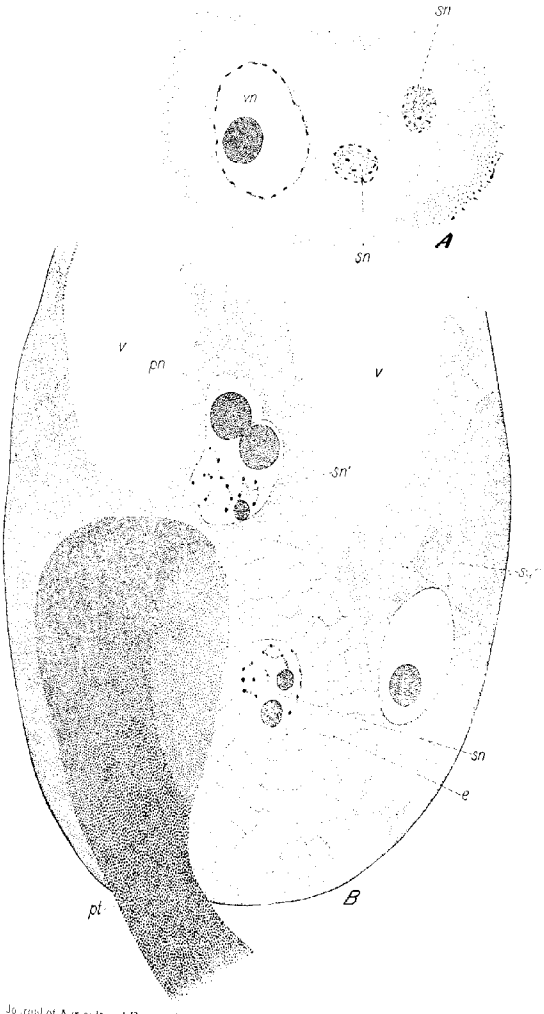
B.—Cross section of a fibro-vascular bundle of the silk: x, xylem elements; sc, sheath cells; pt, pollen tube. × 650.

C.—Longitudinal section through the sheath cells of the fibro-vascular bundles: sc, sheath cells; pc, parenchyma cells of the silk. It is between the sheath cells that the pollen tube works its way down the silk. × 650.

PLATE 31

A.—Vegetative and sperm nuclei of the pollen grain: vn, vegetative nucleus; sn, sperm nuclei. × 1,100.

B.—Longitudinal section of the lower portion of the embryo sac at the time of fertilization, reconstructed from two sections: pn, polar nuclei fusing; sn′, sperm nucleus fusing with a polar nucleus; e, egg; sn, sperm nucleus in the egg; pt, pollen tube; syn, synergid; v, vacuole. × 1,100.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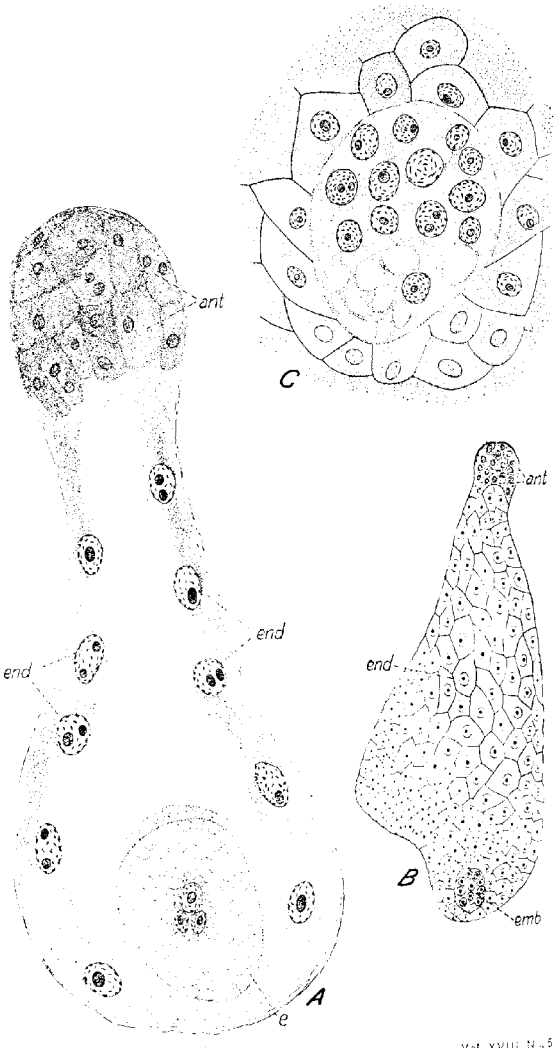
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PLATE 32

A.—Longitudinal section of the embryo sac 12 hours after fertilization: end, endosperm nuclei; e, egg in which one of the daughter nuclei has already divided; ant, antipodals. × 520.

B.—Longitudinal section of the embryo sac 36 hours after fertilization: end, endosperm; emb, embryo; ant, antipodal tissue. × 110.

C.—Longitudinal section of the young embryo at the stage shown in B. × 800.

RESPONSE OF CITRUS SEEDLINGS IN WATER CULTURES TO SALTS AND ORGANIC EXTRACTS

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INTRODUCTION

This paper deals with the growth of citrus seedlings in water cultures and their reaction to various salts and to organic matter in solution. The experiments were conducted mainly at Riverside, Calif., in connection with other investigations relating to the causes of the malnutrition of citrus trees. Lemon, grapefruit, and several varieties of orange seedlings (Blood, Tahiti, Mission, Valencia, Tangerine) were used in the experiments; but the work was confined largely to grapefruit and lemon on account of the relative ease with which the seeds could be secured.

EXPERIMENTAL METHOD

The seeds were sprouted in trays of coarse sand about 10 cm. deep and were covered to a depth of about 1 cm. At ordinary spring and summer temperatures it requires about 30 days to develop citrus seedlings to a stage suitable for culture work, but under greenhouse conditions this time can be reduced to about 20 days. The germination period of the grapefruit and lemon is somewhat less than that of the orange. The seeds should not be given much water during the germinating period. The citrus seedling is hardy and will stand drouth. Hot sunshine is likely to scald the primary leaves, so it is advisable to keep the seedlings in half shade.

When the seedlings were large enough to be used, the sand around the roots was saturated with water and the seedlings were withdrawn from the seed bed. The sand was washed off and the roots dipped into a thick paste of carbon black to a depth of about 1 cm. The carbon black sticks to the radicle and forms a well-defined index, so that the subsequent elongation of the root tip can be accurately measured. Five to 10 seedlings bound together lightly with a small piece of absorbent cotton were usually placed in each culture flask. Of the citrus seedlings so far tested, the lemon is by far the most satisfactory for use in water cultures, for the radicle is straight and the cotyledons do not separate as in the grapefruit.

It was found that the citrus seedling is especially sensitive to the toxic substances in distilled water. A treatment with carbon black that would

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purify the water sufficiently for wheat seedlings was inadequate for the citrus seedling. It was necessary to use an extra large amount of carbon black or to bring the water to the boiling point in the presence of carbon black and filter in order to obtain water in which citrus seedlings would grow. Individual variation in the resistance of the seedlings was frequently observed. In a toxic solution that would kill nine of the seedlings, the tenth would sometimes grow vigorously.

ORGANIC EXTRACTS

In the early experiments organic extracts from acid upland peat (leaf mold) and horse manure were employed. The organic matter was first extracted to obtain the water-soluble portion and then extracted with 3 per cent ammonia. These extracts were filtered and evaporated separately to dryness and the dried material used to make up standard organic solutions. The peat extract proved to be fully as satisfactory for cultures as that from manure and has been employed in most of the work.[1]

The effect of organic matter upon the growth of the lemon seedling is shown in Table I. The result in each instance represents the total root growth of 10 seedlings in six days. It will be seen that the water-soluble extracts of peat in concentrations of 10 parts per million or more produced root elongation double that produced with cultures in carbon-treated water.

TABLE I.—*Effect of peat extract on root elongation of citrus seedlings*

Treatment.	Root growth.
	Mm.
Carbon-treated water, control	90
Carbon-treated water, plus 5 p. p. m. water-soluble peat	115
Carbon-treated water, plus 10 p. p. m. water-soluble peat	197
Carbon-treated water, plus 50 p. p. m. water-soluble peat	197
Carbon-treated water, plus 100 p. p. m. water-soluble peat	236
Carbon-treated water, plus 500 p. p. m. water-soluble peat	178
Carbon-treated water, plus 1,000 p. p. m. water-soluble peat	190

A similar series of experiments with grapefruit seedings, carried on at the same time, gave results almost as marked. The root growth in the two cultures is illustrated in Plate 33, A, B.

The stimulating effect of the soluble organic matter in very low concentrations was verified by repeated tests and was observed not only for grapefruit and lemon seedlings but for Blood, St. Michael, Tahiti, Valencia, and Tangerine oranges as well. The ammonia extract of the peat, freed from ammonium hydrate (NH_4OH) by evaporating to dryness, was as effective as the water-soluble extract in stimulating the root growth of the seedlings.

[1] This peat is the same as that employed by Coville in his investigation of blueberry culture. (COVILLE, F. V. EXPERIMENTS IN BLUEBERRY CULTURE. U. S. Dept. Agr. Bur. Plant Indus. Bul. 193, 100 p., 3[illegible]

The organic matter extracted from upland peat is decidedly acid, but the degree of acidity varies greatly in different samples. In the upland peat employed in this work, 1 gm. of the soluble material extracted with 3 per cent ammonium hydrate, after being evaporated to dryness to remove the ammonia and again dissolved in water free from carbon dioxid, required 0.088 gm. sodium hydroxid to neutralize it, using phenolphthalein as an indicator. The acidity is therefore about 8 per cent of an hydrochloric acid solution of the same concentration by weight. Since the root elongation was stimulated in the most concentrated organic extracts used (1,000 parts per million), it appears that at least in the presence of the other constituents of the peat extract the growth of the citrus seedlings is not inhibited by the organic acids present in concentrations equivalent to 80 parts per million hydrochloric acid.[1]

The figures in Table I, showing the root elongation, are not to be considered as representing accurately the relative stimulation of the various peat concentrations. Often a solution of 10 parts per million of peat would give as good plants as a solution of higher concentration. No attempt was made to find the amount of organic matter needed for maximum growth. Neither potassium chlorid nor sodium nitrate in water cultures stimulated the growth of the seedlings, so the action of the organic matter is not to be attributed to the addition of these nutrient salts.

STIMULATING ACTION OF CALCIUM CARBONATE AND BICARBONATE

Calcium carbonate was found to have as pronounced an effect as organic matter in stimulating the root elongation of citrus seedlings. This is shown in the following table, the figures representing the total root growth of 10 seedlings in eight days. About 0.1 gm. of calcium carbonate was added to each culture flask, which contained about 250 cc. of solution.

TABLE II.—*Stimulation of root growth of citrus seedlings by calcium carbonate*

Seedling.	Root growth in—	
	Carbon-treated water.	Carbon-treated water plus calcium carbonate.
	Mm.	*Mm.*
Lemon	92	435
Grapefruit	100	275

[1] The nature of the organic acids present in the upland peats of the United States does not appear to have been specifically investigated. Some of the organic acids and other substances of definite chemical composition which have been isolated from alkaline extracts of soils are listed in a paper by Shreiner and Shorey, who state that the vegetable acids of the hydroxy-fatty series which are added to the soil with the plant residues apparently soon break down into simpler compounds. (SCHREINER, Oswald, and SHOREY, Edmund C. CHEMICAL NATURE OF SOIL ORGANIC MATTER. U. S. Dept. Agr. Bur. Soils Bul. 74, p. 13. 1910.)

The stimulating action of calcium carbonate was observed with all the different kinds of seedlings studied. Its effect on lemon and grapefruit seedlings is illustrated in Plate 33, C–F.

Carbon dioxid was passed through carbon-treated water containing an excess of calcium carbonate. The resulting solution was found upon analysis to contain over 400 parts per million calcium as calcium bicarbonate. After portions were withdrawn and slightly agitated to expel the excess of carbon dioxid, citrus seedlings were introduced into the solution. With all the seedlings tried a stimulating effect was noticeable.

AMELIORATING ACTION OF LIME AND ORGANIC MATTER WHEN ADDED TO TOXIC SOLUTIONS

A slightly toxic solution was prepared by mixing one part of untreated distilled water with three parts carbon-treated water. This gave a solution that would stop the root development of all the seedlings. The addition of calcium carbonate (solid phase present) and organic matter to this solution invariably enabled the plant to overcome the toxic effect of the solution. Many tests of this kind were made with all kinds of citrus seedlings. A fair example of the relative root development of a 12-day-old culture of grapefruit seedlings is shown in Table III.

TABLE III.—*Protective action of lime and organic extracts*

Treatment.	Root development.
	Mm.
Toxic water, control	0
Toxic water, plus 20 p. p. m. organic matter	145
Toxic water, plus calcium carbonate (solid phase present)	148

Calcium is known to have a marked antagonism to the toxicity of some inorganic salts. The protective and stimulating action of the calcium carbonate is, however, in this instance not explainable on this basis. Citrus seedlings showed no growth in toxic water mixtures to which 10 parts per million of calcium sulphate or of calcium chlorid had been added. The solubility of calcium carbonate in distilled water is approximately 10 parts per million. From the fact that calcium chlorid and calcium sulphate in the same concentration showed no protective action it appears that it is not the calcium ion itself that gives rise to the antagonistic action on distilled water toxins. Furthermore, the calcium content of the organic dried peat extract was only 0.3 per cent, so that protective action of the peat in concentrations of 20 parts per million is not attributable to the calcium it contains. Though the nature of the protective action has not been determined, the adsorption of the toxins on the calcium carbonate particles present in the solid phase and by the colloidal organic constituents is suggested as a possible explanation.

TOXIC LIMITS OF ALKALINE SALTS

The toxicity of calcium hydrate, sodium hydrate, and sodium carbonate was determined for lemon and grapefruit seedlings which showed practically the same degree of resistance. The cultures in calcium hydrate solutions while under observation were kept in a large desiccator containing quicklime. A concentration of 25 parts per million of calcium hydrate or sodium hydrate gave stimulating results. When the concentration of calcium hydrate was increased to 50 parts per million, signs of distress were noted. Little growth took place at 80 parts per million, and at 100 parts per million growth practically ceased. Here a wide variation was noted among the seedlings. Occasionally a vigorous seedling would withstand a concentration of 120 parts per million of calcium hydrate. This was exceptional, however, and it may be said that the citrus seedling will seldom tolerate 100 parts per million of calcium hydrate.

With sodium hydrate a concentration of 25 parts per million stimulated the root growth, and concentrations up to 200 parts per million were maintained with little harmful effect upon the plant. With 250 parts per million the growth was slight, while with 275 to 300 parts per million growth practically ceased. With sodium carbonate it was necessary to increase the concentration to 550 or 600 parts per million in order to stop the growth of the seedlings. It is of interest to note that a solution of this concentration would, on account of hydrolysis, contain sodium hydroxid in approximately the same concentration as that representing the toxic limit of sodium hydroxid. In other words, it is the hydrolyzed portion of the sodium carbonate which mainly determines the toxicity.

Since calcium hydrate and sodium hydrate have nearly the same equimolecular weights, it follows that the hydroxyl concentration in the toxic calcium hydrate solution is only about one-third that in the toxic sodium hydrate solution. It is evident that the metallic ion is contributing also to the toxicity.

When organic matter (extracted from peat with ammonia) which is acid in reaction and stimulating to root growth is added in the proportion of 100 parts per million to a solution containing 400 parts per million of sodium carbonate, a toxic body is formed that will kill the root tip of the seedlings. (Pl. 34, A–D.) This is not true, however, with organic matter extracted from peat with water. This class of reactions appears to be of importance in connection with the toxicity of alkaline soils and will be made the subject of a later report.

TOXIC LIMITS OF ACIDS

The toxic properties of only one inorganic acid, phosphoric acid, were investigated. This acid stopped the root development at a concentration of 20 parts per million, with both the grapefruit and the lemon. Attention has already been called to the fact that active root elongation takes place in organic extracts from peat having an acidity equivalent to 80 parts per million of hydrochloric acid. In overripe lemons the seeds are likely to germinate inside the fruit and the plumule and radical be pushed through the skin. Since the juice of the lemon contains 7 or 8 per cent citric acid, it appears that under natural conditions the lemon seeds will sprout in a strongly acid medium.

TOXIC LIMITS OF NITRATES AND OF AMMONIUM SULPHATE

It is known that the continued use of sodium nitrate in relatively large amounts tends to produce mottling of the leaves of citrus tress. Culture tests were accordingly made to determine the toxicity of the nitrate salts together with that of ammonium sulphate, which is also used in citrus districts as a source of nitrogen. The results are given in the following table, with the toxic limits of the same salts in the presence of lime.

TABLE IV.—*Toxic limits of nitrates and ammonium sulphate for citrus seedlings*

Salt.	Toxic limit.
	P. p. m.
Sodium nitrate	1,800
Potassium nitrate	3,500
Calcium nitrate	10,000
Ammonium sulphate	1,000
Sodium nitrate and calcium carbonate (solid phase)	6,000
Ammonium sulphate and calcium carbonate (solid phase)	2,000

It will be seen that marked differences occur in the toxic limits of the various salts, sodium nitrate being five times as toxic as calcium nitrate. The toxic limits for this group of salts are so high that the matter may appear to be of no practical import. But a simple calculation will show that the surface feeding roots of citrus trees are at times subjected to fertilizer concentrations in field practice so great as to approach toxic conditions. Application of 2 to 3 pounds of nitrate of soda per tree, or 200 to 300 pounds per acre, which is not an unusual practice for some citrus growers, would correspond approximately to a concentration of 70 to 100 parts per million in the soil of the surface foot. The fertilizer, moreover, is ordinarily applied to the open ground between the tree rows—that is, to not more than one-half the total soil area. If the moisture content of the soil were reduced to 10 per cent of the weight of the soil, the concentration of the sodium nitrate in the soil solution would range

from 1,400 to 2,000 parts per million—that is, it would approach the toxic limit. The surface crusts in citrus groves are often highly toxic to citrus seedlings.

However, mixed salts in the soil are not as a rule so toxic as the individual components. The protective or antagonistic action of calcium, for example, when added to toxic solutions of many inorganic salts is well known. Kearney and Cameron [1] found that gypsum and lime greatly increased the tolerance of white lupine and alfalfa seedlings for most of the salts found in saline soils. Osterhout has observed antagonism in many mixtures of salts and has shown that, in general, if one salt increases and the other decreases the permeability of the protoplasm, as determined by electrical conductivity, then the two salts tend to be antagonistic.[2] He has also found that while none of the monovalent ions, excepting hydrogen, decrease permeability, all the bivalent ions tested (calcium, magnesium, barium, etc.) do so to a marked degree.[3] In other words, the two groups are antagonistic.

It was consequently to be expected that calcium carbonate would increase the tolerance of citrus seedlings for sodium nitrate or potassium nitrate. The effect was most pronounced with a mixture of sodium nitrate and calcium carbonate (solid phase present) in which a concentration of 6,000 parts per million of sodium nitrate, or over three times that with sodium nitrate alone, was reached before the death of the root tip occurred (Pl. 34, E–G). With ammonium sulphate the death limit in the presence of calcium carbonate was reached at a concentration of about 2,000 parts per million, or twice that with ammonium sulphate alone. The filtrate obtained after shaking the higher concentration of sodium nitrate with calcium carbonate in excess and removing the solid phase produced as good plants as when the solid phase was present. The mechanical or adsorptive action of the latter, therefore, has no effect. The addition of small quantities of quartz flour, carbon black, or organic matter to the solution was also without effect on the toxic limit of the nitrate solutions.

When calcium nitrate or calcium sulphate in concentrations equivalent to the saturation concentration of calcium carbonate in water (10 parts per million) was added to sodium nitrate solutions, the toxic limit was not raised appreciably above that of sodium nitrate alone. As the concentrations were increased, the protective action developed; and in concentrations of 100 parts per million calcium chlorid or calcium sulphate was about as effective as calcium carbonate in raising the toxic limit of sodium nitrate. On account of the reaction between sodium nitrate and

[1] KEARNEY, Thomas H., and CAMERON, Frank K. EFFECT UPON SEEDLING PLANTS OF CERTAIN COMPONENTS OF ALKALI SOILS. *In* U. S. Dept. Agr. Bul. 71, p. 7–00. 1902.

[2] OSTERHOUT, W. J. V. ON THE NATURE OF ANTAGONISM. *In* Science, n. s. v. 41, no. 1050, p. 255–256, 1914.

[3] OSTERHOUT, W. J. V. ON THE DECREASE OF PERMEABILITY DUE TO CERTAIN BIVALENT KATIONS. *In* Bot. Gaz., v. 59, no. 4, p. 317–330, 11 fig. 1915.

calcium acrbonate when the latter salt is present in the solid phase, the calcium-ion concentration is considerably increased above that of a saturated solution of calcium carbonate alone. This increase in the concentration of the calcium ion, which gives the protective action, is probably the explanation of the failure of calcium chlorid and calcium sulphate to exert a protective action at concentrations corresponding to the solubility of calcium carbonate alone. Calcium nitrate and calcium hydrate in small amounts were also as effective as calcium carbonate. Organic matter had no ameliorating effect on the toxic action of sodium nitrate.

SUMMARY

Seedlings of various citrus stocks, including lemon, grapefruit, and several varieties of sweet oranges, showed no characteristic differences in response in water cultures or in resistance to toxic solutions.

Very dilute organic extracts from upland peat (10 parts per million or more) produced a marked stimulation of the root growth of citrus seedlings. Corresponding concentrations of sodium nitrate or potassium chlorid did not stimulate the root development.

Calcium carbonate stimulated the root growth and exerted a pronounced antagonistic action to toxic solutions of nitrates and ammonium sulphate.

Peat extract in very dilute concentrations (20 parts per million) and calcium carbonate (solid phase present) both protected citrus seedlings to a marked degree against the toxins of distilled water.

The tolerance of citrus seedlings for alkaline salts is relatively high. The toxic limit for calcium hydrate was 100 to 120 parts per million, for sodium hydrate 250 to 300 parts per million, and for sodium carbonate 550 to 600 parts per million. The hydroxyl concentration in the toxic calcium hydrate solution is only about one-third that of the toxic sodium hydrate solution.

When soluble organic matter which is acid in reaction and stimulating to citrus seedlings in concentrations up to 1,000 parts per million or more is added to a sodium carbonate solution of 400 parts per million which in itself is not toxic, a highly toxic solution is formed which will kill the root tips of citrus seedlings. This reaction appears to be of importance in connection with the toxicity of soils containing small amounts of sodium carbonate.

PLATE 33

A.—Grapefruit roots, 12 days old, grown in distilled water.

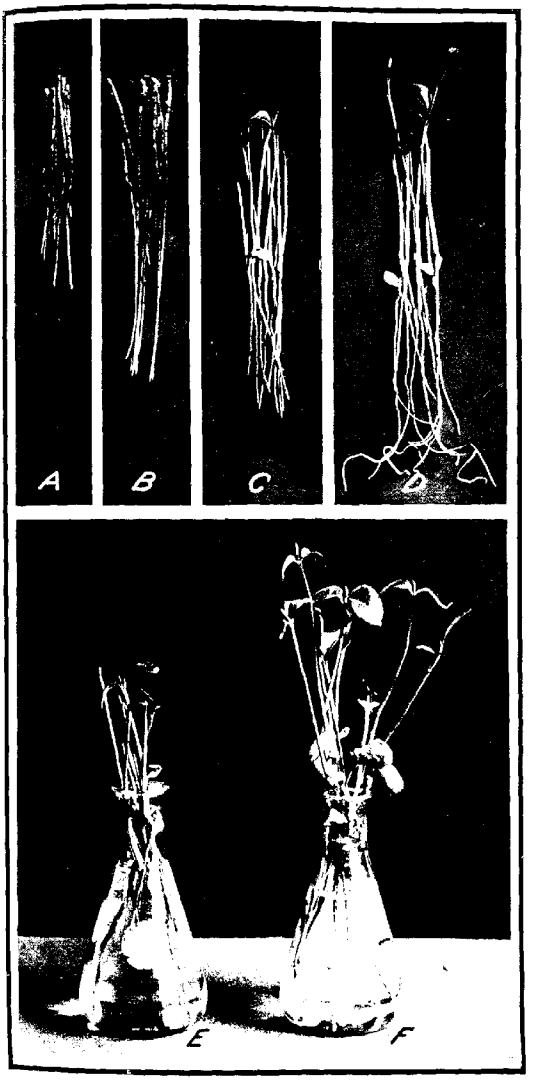
B.—Grapefruit roots, 12 days old, grown in distilled water plus 100 parts per million water-soluble peat.

C.—Lemon seedlings, 21 days old, grown in carbon-treated distilled water.

D.—Lemon seedlings, 21 days old, grown in carbon-treated distilled water plus calcium carbonate.

E.—Grapefruit seedlings, 20 days old, grown in carbon-treated water.

F.—Grapefruit seedlings, 20 days old, grown in carbon-treated water plus calcium carbonate.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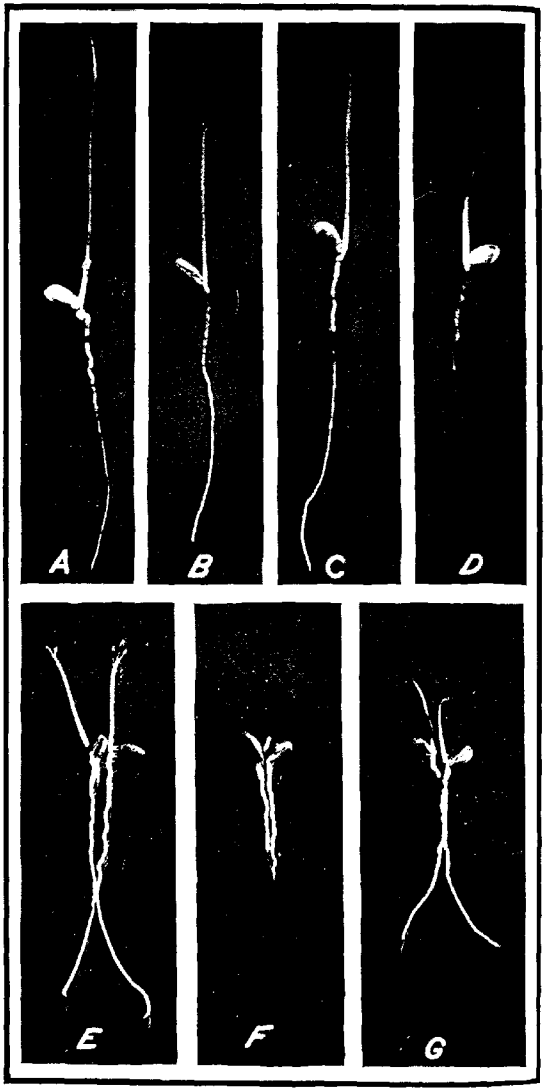
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PLATE 34

A.—Lemon seedlings, 16 days old, grown in distilled water as control.

B.—Lemon seedlings, 16 days old, grown in 400 parts per million sodium carbonate.

C.—Lemon seedlings, 16 days old, grown in 100 parts per million ammonia-soluble humus.

D.—Lemon seedlings, 16 days old, grown in 100 parts per million ammonia-soluble humus plus 400 parts per million sodium carbonate.

E. —Citrus seedlings grown in carbon-treated water.

F.—Citrus seedlings grown in 4/10 per cent sodium nitrate ($NaNO_3$).

G.– Citrus seedlings grown in 4/10 per cent sodium nitrate with calcium carbonate ($CaCO_3$).

PHYSIOLOGICAL STUDY OF THE PARASITISM OF PYTHIUM DEBARYANUM HESSE ON THE POTATO TUBER

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INTRODUCTION

The physiology of parasitism and the relations existing between the host and parasite have been the subject of numerous investigations, many of which have taken up the method by which the fungus obtains entrance into the plant or passes from cell to cell within the tissues of its host.

There are, of course, several possible ways by which a parasitic plant may obtain entrance into the cells of its host plant. It may enter through an opening already made; if it makes the opening itself, it may push its way through mechanically, or it may soften or digest the cell walls. It is possible, also, that the fungus hyphae might so stimulate the cells of the host plant that enzyms secreted by the host itself would break down its own cell walls and allow the fungus to enter. A combination of these methods is, of course, possible—for example, a fungus might penetrate the cell wall by a small opening and then enlarge this opening either mechanically or by a solution of a portion of the cell wall. Some of the investigations on this subject will be considered here.

In 1886 De Bary (*3*)[1] showed that *Sclerotinia libertiana* secreted a toxic substance which killed the cells ahead of the growth of the fungus. He concluded that the breaking down of the cell walls was due to an enzym secreted by the fungus.

Ward (*27*) concludes, in his study of Botrytis on lily, that the tip of the fungus hypha "excretes relatively large quantities of ferment substance and dissolves its way into the cell wall." Nordhausen (*21*) considers that *Botrytis cinerea* dissolves its way through the cell walls of its host plant. Büsgen (*9*) considers that this fungus does not make its way through cell walls or even cuticle by mechanical means alone. Miyoshi (*20*) showed that *Botrytis cinerea* could force its way through membranes of collodion, paper, and other substances, and details some experiments in which Penicillium pushed its way through gold leaf. Peirce (*23*) has shown that the haustoria of Cuscuta will puncture tinfoil 0.2 mm. in thickness.

[1] Reference is made by number (italic) to "Literature cited," p. 295-297.

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Brown (*6*) in a recent study of parasitism in Botrytis has shown that this fungus secretes an enzym which breaks down the middle lamellae of tissues which it invades. He demonstrated that the enzym secretion was more powerful from freshly germinated spores than from old cultures. A toxin, which is apparently closely associated with the enzym, is also secreted. This toxin is not oxalic acid or an oxalate. Blackman and Welsford (*5*), in the second of this series of papers, have shown that this fungus apparently penetrates the cuticle of the broad bean leaf by pushing its way through mechanically. These writers are in agreement with Brown that an enzym secreted by the fungus breaks down the tissues in the interior of the leaves. Brown (*7*) later showed that the infecting germ tubes were unable to affect chemically the cuticle of the host plant and that the toxic substance could not pass through the cuticle. He concludes that penetration of the cuticle must take place in a purely mechanical way. In the fourth paper of this series (*8*) he contrasts thick and thin sowings of spores and finds that thick sowings 2 to 4 days old yield the most active preparations of enzym. He considers that the cytase is much more active at the tip of the hypha.

From this review, which of course covers only part of the literature on this subject, it is apparent that there is good evidence that some parasitic plants make their way into their host plants by breaking through the tissues mechanically; but there is no doubt that some fungi secrete enzyms which break down the cell walls of certain plants and are thus able to make their way through the tissues of their hosts.

The parasitism of *Pythium debaryanum* Hesse on some of its numerous hosts has been investigated, but how it gains entrance into the host plant seems not to have received any considerable attention. This fungus has recently been shown to be the cause (*15*) of a tuber-rot of potatoes which is of considerable commercial importance in the San Joaquin Valley of California. A method of controlling this disease under commercial conditions has been worked out and described (*17*). In the present study the effect of the fungus on the sugars, pentosans, and starch of the potato tuber was determined, and the rate of growth of the fungus in three different varieties of potatoes was measured. An attempt was made to correlate certain physical and chemical characteristics of the potatoes with their susceptibility or resistance to this disease, and the growth of the fungus in the potato tissue was observed and studied. Some information on the mode of entrance of this fungus into the cells of the potato was obtained, and a possible explanation was found as to why some varieties of potatoes are much more susceptible to this disease than others.

EXPERIMENTAL WORK

The methods followed in the study of the effect of the fungus on the starch, sugars, and pentosans of the potato tuber were essentially those described for the work with the Fusarium tuber-rots (*16*). They will not be discussed here. The results of the analyses of the sound and rotted quarters are shown in Table I.

TABLE I.—*Starch, sugar, and pentosan content of the sound and rotted quarters of potatoes rotted with Pythium debaryanum*

[Expressed as percentage of wet weight]

Pentosans.			Starch.			Sugar.				
						Sucrose as dextrose.			Reducing sugar as dextrose.	
Tuber No.	Sound quarter.	Rotted quarter.	Tuber No.	Sound quarter.	Rotted quarter.	Tuber No.	Sound quarter.	Rotted quarter.	Sound quarter.	Rotted quarter.
1.....	0.548	0.44	5....	16.07	14.64	9...	0.98	0.030	0.220	0.003
2.....	.39	.32	6....	16.61	16.34	6...	.63	.009	.252	.002
3.....	.33	.27	7....	16.73	13.04	10...	.11	.008	.392	.012
4.....	.35	.31	8....	15.65	11.42	11...	.46	.020	.440	.001

In Table I it is noticeable that the sugars, both sucrose and reducing sugars, had almost disappeared in the rotted portions of the tuber, while appreciable amounts were present in all the uninfected quarters. The fungus is evidently able to utilize the sugars of its host. In this its action is similar to that of *Fusarium oxysporium*, *F. radicicola*, and *F. coeruleum* (*13*) on potatoes, *Sclerotinia fructigenia* on apples (*4*) and peaches (*14*), *Sphaeropsis malorum* on apples (*12*), and *Rhizopus nigricans* (*25*) on strawberries, all of which cause a decrease in the sugar content of the host plant or part of the host invaded. The results with these several fungi seem to justify the conclusion that rot-producing fungi are usually able to break down and utilize the sugars of the host.

The starch content of the potato also decreases when rotted by *Pythium debaryanum*, as is shown in columns 5 and 6 of Table I. Starch grains were frequently found corroded; and an extract of the fungus mycelium, which had been grown on either potato plugs or mashed potatoes which had been sterilized, was able to pit potato starch grains as well as to digest gelatinized potato starch. In this respect the rot produced by this fungus is different from that produced by any of the three species of Fusarium mentioned above. With the Fusarium-rots no corrosion of the starch grains was noticeable, and the starch content of the rotted portions was not lower than that of the corresponding sound quarter. An extract of the mycelium of any of these three fungi was apparently incapable of corroding grains of potato starch even

after a long period, though gelatinized potato starch and soluble starch were readily digested by the extracts. The results obtained with *P. debaryanum* on potato starch are not in accord with the findings of Ward (*26*), who concluded that this fungus did not attack the starch of the potato. His conclusions were based entirely on microscopical observations. The pentosan content of portions of the tuber-rots by *P. debaryanum* is somewhat lower than that of the corresponding sound quarters, and from this it may be concluded that the fungus is able to digest the pentosans. This conclusion is supported by the fact that in potatoes rotted by this organism the middle lamellae of the cells are broken down and the cells may be readily teased apart on a slide. Extracts of the mycelium also digest the middle lamellae, and a slice of potato ½ mm. in thickness disintegrates in 12 hours when immersed in it. The middle lamellae, however, seem to be the only portion of the cell wall affected, for when a bit of rotted tuber is placed on a slide and teased out the cells float free, while only in exceptional cases are broken cells seen. The fungus penetrates the tissue in all directions but seems most frequently to pass directly through the cell wall.

In inoculation experiments with this fungus it was found that Bliss Triumph and Green Mountain potatoes were very susceptible to this disease, while the White McCormick potatoes were not. In the experiments all the Bliss Triumph and Green Mountain tubers rotted eventually—90 per cent as a result of the first inoculation—while with the McCormicks only about 30 per cent of the potatoes seemed to be susceptible to the disease even when inoculated three different times. When a McCormick tuber did become infected, the rot usually developed very slowly. This variety, while not immune to the disease, seemed to be highly resistant.

Measurements of the rate of growth of the fungus were made in tubers of the three different varieties mentioned. The method followed was to cut cylinders of the potato tubers about 12 mm. in diameter and 30 mm. long. These cylinders were placed on end in a small moist chamber and inoculated on the upper end from stock cultures of the fungus. After incubating for 24 hours the cylinders were sliced transversely into sections 1 mm. in thickness. These slices were numbered in order and placed in a moist chamber. The slices in which the rot developed were noted, and it was thus possible to determine within a millimeter the distance the fungus had progressed in 24 hours. This method is somewhat similar to the method followed by Jones, Giddings, and Lutman (*18*) in their study of the resistance of potatoes to *Phytophthora infestans*. The rapid rate of growth of the fungus used in the present study, however, made it possible to simplify the method considerably. The results of these experiments are shown in Table II.

TABLE II.—*Average rate of growth of Pythium debaryanum in tissue of Bliss Triumph, Green Mountain, and McCormick potatoes*

Experiment No.	Green Mountain.		Bliss Triumph.		McCormick.	
	Number of cylinders.	Average growth per hour.	Number of cylinders.	Average growth per hour.	Number of cylinders.	Average growth per hour.
		Mm.		*Mm.*		*Mm.*
1	6	0.354	7	0.430	7	0.071
2	9	.458	9	.425	8	.187
3	7	.290	8	.453	8	.049
Average		.366		.436		.102

It is noticeable in Table II that the rate of growth of the fungus in Bliss Triumph and Green Mountain tubers is from three to four times as rapid as in McCormick under the conditions of the experiment. In some cases the fungus was apparently unable to affect the cylinders from the McCormick and had not, at the end of the experiment, penetrated into the first millimeter of tissue. Other cylinders from this variety were much more susceptible, however, so that the average rate of growth of the fungus, as shown in the table, is fairly high.

In order to relate this rate of growth to the number of cells traversed, measurements of the cells in the cortex and central portions of tubers of these three varieties were made. About 1,500 measurements were made with each variety. The averages for the cortex and central portions of the three varieties are given below:

TABLE III.—*Average size of cells in the three varieties of potatoes from 1,500 measurements on each variety*

	Bliss Triumph.	Green Mountain.	McCormick.
Cortex	269μ × 303μ	294μ × 311μ	269μ × 303μ
Central portion	318μ	318μ	347μ

If it is considered, then, that the same rate of growth holds for the cortex and interior of the tuber, the average length of time required for the fungus to pass through an average cell in the interior would be 43, 39, and 204 minutes, respectively, for the three varieties, Bliss Triumph, Green Mountain,[2] and McCormick. The fact that the cells are so nearly of the same size in the three varieties would eliminate the possibility that the relatively slow rate of growth of the fungus in the McCormick tubers was due to the small size of the cells and the consequently larger number

[2] In a former paper (17) one of the writers gives the size of the cell in a Green Mountain tuber as 138μ, which is erroneous. The tuber mentioned was of the Burbank variety. The size of the cells and rate of growth given in the present paper are correct for this variety.

of cell walls for the fungus to penetrate in traveling a given distance. It may, however, be due to some resistant quality of the cell wall.

That the fungus secretes a toxic substance which kills potato cells was demonstrated experimentally by a method somewhat similar to that followed by Brown (*6*, *7*, *8*) in his work with *Botrytis cinerea*. Cultures of the fungus were grown for two weeks on sterilized potato mush, and potato plugs and the mycelium were removed in such a way that none of the culture medium adhered to the mat of mycelium. The mycelium was then ground in a mortar with sand, extracted with distilled water, and filtered. Cylinders about 1 cm. in diameter were cut from potato tubers and sliced into disks 0.5 mm. in thickness. Some of these disks were placed in the extract of mycelium and some in distilled water and examined at intervals. After three hours the disks in the fungus extract had lost their turgidity so that when grasped by the edge with a pair of forceps and held in a horizontal position they collapsed limply. The disks from the distilled water preparation remained turgid for 12 hours or more. Disks from the preparation of fungus extract did not resume their normal turgidity when washed and placed in distilled water. The cells of the potato are apparently killed by some substance extracted from the ground mycelium. The loss of turgidity can not be accounted for by a loss of water from the potato cells caused by a higher osmotic pressure in the extract, because tests showed that the lowering of the freezing point of the extract used was only about one-fifth that of the juice from the potato tuber. All three varieties of potatoes used in these experiments behaved in the same way. From these experiments it seems hardly probable that resistance to fungus attack can be due directly to the living protoplasm.

The macerating effect of this extract on the potato tissue has been mentioned earlier in this paper. The properties of the toxic substance secreted by the fungus were not determined, though the problem is well worthy of investigation.

It has been shown in some cases that resistance to certain fungus diseases was correlated with higher acidity of the host plant tissues. Thus Averna-Sacca (*2*) has shown that the resistance to diseases of grapes caused by Oidium and Peronospora was correlated with a relatively high acidity. Comes (*11*) has demonstrated that a variety of wheat (Rieti), resistant to rust, has an acidity considerably higher than the varieties in the same locality which are susceptible to this disease. Further, this writer has shown that when this resistant variety is grown in other localities where the environmental conditions tend to produce a plant of lower acidity, the plant is susceptible to the disease. These researches indicate that acidity may play a very important rôle in the resistance of a plant to disease.

There are, of course, many other factors that tend to influence the resistance or susceptibility of a plant to disease. The literature on this

subject has been ably reviewed in the papers of Ward (*28*), Appel (*1*), Orton (*22*), and Butler (*10*) and will not be considered in this paper except as it relates directly to the problem.

Inasmuch as *Pythium debaryanum* is rather susceptible to acids, it was considered worth while to test the acidity of two of the varieties of potatoes used in these experiments—Bliss Triumph, which is very susceptible to the disease, and McCormick, the variety which had proved rather resistant. Determinations of hydrogen-ion concentration were made on the expressed juice of tubers of these two varieties by the potentiometric method, and it was found that juice from the McCormick potatoes had a C_H 8.67 × 10^{-6} while that from Bliss Triumph had a C_H 8.63 × 10^{-7}. The McCormick had a hydrogen-ion concentration of about 10 times that of Bliss Triumph. To obtain further evidence on this point the fungus was grown in a series of potato-juice cultures made up to known hydrogen-ion concentration with $N^1/_{100}$ sodium phosphate buffer mixture. The results of these experiments are shown in Table IV.

TABLE IV.—*Growth of Pythium debaryanum in potato juice of various hydrogen-ion concentrations*

C_H of culture medium.	Behavior of fungus.
3.936 × 10^{-9}	No growth in 3 days.
5.035 × 10^{-8}	1 grew in 3 days.
1.738 × 10^{-7}	2 grew well in 3 days.
1.660 × 10^{-6}	Growth covered plates in 3 days.
9.84 × 10^{-6}	Do.
2.535 × 10^{-5}	Do.
5.585 × 10^{-5}	Do.
3.741 × 10^{-4}	Do.
8.69 × 10^{-4}	5 mm. growth in 3 days.

According to Table IV the fungus grows well and fruits in a C_H 3.741 × 10^{-4}, which is considerably higher than that of the McCormick potato. The resistance of the McCormick potato to this disease, then, is not due to its high acidity. This is in accordance with the conclusions of Jones, Giddings, and Lutman (*18*) in regard to resistance of potatoes to *Phytophthora infestans*.

The experiments described in the foregoing pages seemed to indicate that the resistance to the progress of the fungus in McCormick potatoes might be due to some property of the cell wall—that is, it is possible that the fungus makes the opening in the cell wall through which it passes mechanically. If this is true, cell walls of potatoes resistant to the disease should show a higher resistance to puncture by mechanical means than the cell walls of susceptible varieties. This hypothesis seemed worth testing out, so an apparatus for measuring the pressure necessary to puncture the tissue of a potato was arranged.

This apparatus (Pl. 35)[3] consisted of a modified Joly balance, accurately graduated, and with a vernier for close reading. The lower end of the spring was attached to a metal rod which passed through a short glass tube fixed to the stand of the instrument. Hair lines on both the tube and rod made it possible to determine accurately the point at which the tension on the spring balanced any given weight. Tension was applied to the spring by means of a rack and pinion adjustment. It was possible with this balance to weigh to a milligram, which was well within the limits of experimental error in these determinations. A glass rod, the weight of which was less than the capacity of the balance, was suspended from the pan of the balance, and a small glass needle with a rounded end was attached to the lower end of the glass rod. In operating this apparatus a slice of potato was placed on the stage of the instrument, which was so adjusted that the tip of the needle just touched the surface of the potato when the hair line of the indicator on the spring coincided with the hair line of the fixed indicator of the balance. The tension on the spring was then slowly released by means of the rack and pinion adjustment until a sudden drop of the needle indicated that the tip of the needle had penetrated the tissue. The position of the column that supported the spring was then noted on the graduated scale. The weight required to balance the pull of the spring at this point was determined and subtracted from the weight of the needle. The result was the weight required to push the needle into the potato tissue.

Inasmuch as the needles used in these experiments were always from one-sixth to one-fourth the average diameter of potato cells, it is evident that in most cases at least the needle was pushed through the cell wall and that the weights obtained were a close approximation of the pressure necessary to break through the cell wall. The needles were drawn from small glass tubing over a micro burner and were drawn out in such a way as to leave a relatively heavy shoulder so that the slender portion which was thrust into the potato was not more than a millimeter in length. It was found that long needles of the small size necessary in this work were very easily broken. They were rounded and slightly larger at the end so that friction against the sides of the puncture would be reduced to a minimum. The needles used in these experiments were from 58.3 to 71 microns in diameter. In practice 20 determinations were made on each tuber, 10 in the cortex and 10 in the central portion within the ring of bundles. The weights obtained were averaged for each region; and, since the diameter of the needle was known, the weight required to break through tissue per square millimeter of surface was readily calculated. It was shown in this work by using different needles on the same potato that the weights required to puncture potato tissue were about propor-

[3] The authors are indebted to Mr. H. K. Sloat, of the Division of Illustrations, for photographing the motion pictures, and to Mr. J. F. Brewer, of the Laboratory of Plant Pathology, for preparation of the plates in this article.

tional to the area of the cross section of the needles. The determinations made with different needles are thus comparable.

In the experiments, the results of which are shown in Tables V to VII, inclusive, the potatoes were inoculated first in the cortex. This was done by removing a piece of the potato skin about 1 mm. in thickness and 5 to 10 mm. in diameter. A Van Tieghm cell was cemented to the surface of the potato around this wound with vaseline, and a drop or two of sterilized distilled water was placed within the ring and inoculated with mycelium of the fungus. The top of the cell was then closed with a cover glass and vaseline. The inoculated tubers were then placed in an incubator maintained at 30° C. and examined daily. If the tubers became infected, they were removed before they were more than half rotted. They were sliced through the point of inoculation, the distance the rot had progressed was measured, and the weight necessary to puncture the tissue in the two different regions of the sound portions of the tubers was determined. If the inoculation was unsuccessful, a second inoculation was made in the cortex in the same way as the first; and if this inoculation did not result in an infection the potato was inoculated beneath the cortex in the central part. If the results from this third inoculation were negative, as they usually were with McCormick potatoes, the tuber was considered to be immune, and the weight necessary to puncture the tissue was determined as described above. It is worthy of note that Bliss Triumph and Green Mountain potatoes usually rotted as a result of the first inoculation.

The tables in which the results of these experiments are given show the diameter of the needle in microns and the pressure required in grams per square centimeter to penetrate the tissue of the freshly cut potato in the cortex and central portion, respectively. In every case the numbers given as the pressure required to penetrate the tissue are averages of 10 determinations. The same needle was always used for both the cortex and the central portion. The number of inoculations made on each tuber, the region in which they were made, and the result after the length of time indicated are also given. In Table VII, under "results of inoculation," the term "slight rot" appears. This was used to characterize the results of inoculations when there was a browning and slight softening of the tissue immediately around the point of inoculation which seemed to indicate that infection had occurred. The rot had not progressed a measureable distance, however, and the tuber thus affected was apparently practically immune.

TABLE V.—*Pressure in grams per square centimeter required to puncture tissue of freshly cut surface of Green Mountain potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture cell wall.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
27	71	51,556.4	31,938.2	1	Cortex..	40 mm. rot in 3 days.
28	71	48,248.3	35,648.8	1	...do....	30 mm. rot in 3 days.
29	71	37,747.1	32,072.7	1	...do....	27 mm. rot in 3 days.
30	71	39,390.7	25,500.0	1	...do....	42 mm. rot in 3 days.
31	71	57,571.5	47,774.0	1	...do....	16 mm. rot in 3 days.
65	67	42,812.7	33,344.0	1	...do....	30 mm. rot in 4 days.
66	67	47,566.3	42,667.0	1	...do....	19 mm. rot in 3 days.
67	67	31,132.0	30,160.4	1	...do....	34 mm. rot in 4 days.
68	67	34,439.6	34,749.0	1	...do....	29 mm. rot in 4 days.
69	67	33,540.3	33,902.0	1	...do....	49 mm. rot in 4 days.
70	67	47,344.0	25,736.6	1	...do....	25 mm. rot in 4 days.
72	67	32,372.4	29,457.6	1	...do....	25 mm. rot in 5 days.
73	67	42,965.1	39,525.0	1	...do....	30 mm. rot in 5 days.
74	67	39,008.1	26,367.1	1	...do....	33 mm. rot in 5 days.
75	67	31,276.4	22,843.6	1	...do....	34 mm. rot in 5 days.
80	67	60,103.9	41,953.8	3	Deep....	12 mm. rot in 10 days.
61	67	75,525.1	28,351.7	3	...do....	18 mm. rot in 4 days.
55	68.3	47,897.0	39,152.8	3	...do....	18 mm. rot in 2 days.
Average		48,682.0	31,325.0			
Average for tubers which when inoculated in cortex rotted		40,731.3				
Average for tubers which when inoculated in cortex did not rot		61,175.3				

TABLE VI.—*Pressure in grams per square centimeter required to puncture tissue of freshly cut surface of Bliss Triumph potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture cell wall.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
32	71	40,548.1	30,345.4	1	Cortex..	32 mm. rot in 3 days.
33	71	51,457.7	27,723.0	1	...do....	31 mm. rot in 3 days.
34	71	42,662.5	27,824.6	1	...do....	Do.
35	71	39,380.2	30,650.5	1	...do....	24 mm. rot in 3 days.
36	71	53,028.7	35,401.0	1	...do....	19 mm. rot in 5 days.
39	71	45,953.9	34,946.0	1	...do....	68 mm. rot in 5 days.
40	71	51,726.4	34,264.0	1	...do....	42 mm. rot in 5 days.
41	71	40,332.1	36,610.2	2	...do....	28 mm. rot in 4 days.
42	71	40,579.1	29,934.0	2	...do....	46 mm. rot in 4 days.
43	71	37,798.6	29,892.0	2	...do....	32 mm. rot in 4 days.
62	67	41,447.3	29,365.0	1	...do....	18 mm. rot in 4 days.
63	67	34,501.5	22,504.4	1	...do....	23 mm. rot in 4 days.
64	67	33,282.0	29,819.4	1	...do....	24 mm. rot in 4 days.
71	67	33,282.0	22,677.2	1	...do....	28 mm. rot in 4 days.
56	68.3	45,705.8	41,579.0	3	Deep....	5 mm. rot in 2 days.
57	68.3	40,000.3	30,343.4	3	...do....	Do.
Average		41,980.0	38,209.0			
Average for tubers which when inoculated in cortex rotted		38,998.0				
Average for tubers which when inoculated in cortex did not rot		42,852.9				

TABLE VII.—*Pressure in grams per square centimeter required to puncture the tissue of freshly cut surface of McCormick potatoes and results of inoculating these potatoes with Pythium debaryanum*

Tuber No.	Diameter of needle (in microns).	Pressure required to puncture tissue.		Number of inoculations.	Location of final inoculation.	Results of inoculations.
		Cortex.	Central part.			
45	71	75,957.3	61,375.2	3	Deep	Slight rot in 10 days.
46	71	86,357.1	62,615.5	3	do	Slight rot in 5 days.
47	71	105,644.3	82,429.2	3	do	Do.
48	58.3	112,803.3	67,111.7	3	do	Slight rot in 7 days.
49	58.3	77,189.2	55,835.1	3	do	Slight rot in 5 days.
50	58.3	100,362.2	62,284.9	3	do	No rot in 7 days.
51	58.3	138,360.5	113,923.0	3	do	Do.
53	58.3	67,566.4	51,101.6	3	do	10 mm. rot in 7 days.
54	58.3	78,119.5	54,243.3	3	do	12 mm. rot in 10 days.
83	67	51,824.7	48,083.2	1	Cortex.	35 mm. rot in 8 days.
Average		89,368.4	65,900.2			
Average for tubers which when inoculated in cortex did not rot		93,539.9				
Average for tubers which when inoculated in central part did not rot			72,224.9			
Average for tubers which when inoculated in central part rotted			52,672.4			

Tables V to VII show that there is considerable difference in the pressure required to puncture the tissue of the different regions of tubers of the three varieties used. If the pressures required to puncture the tissues of similar regions in the different varieties are compared, it is evident that the pressure is considerably higher for McCormick than for the two susceptible varieties, Bliss Triumph and Green Mountain, while the averages for the last two mentioned are in much closer agreement.

In regard to susceptibility to infection by the fungus, only 1 McCormick tuber out of 10 became infected when inoculated in the cortex, and the pressure required for puncturing the cortex of this tuber was much below the average required for the central portion of this variety. Two other McCormick potatoes became infected when inoculated in the central part, and these tubers were also lower in their resistance to puncture in this region than the others.

Three tubers of Green Mountain potatoes did not become infected even when inoculated twice in the cortex. The average pressure required for the cortex of these three tubers is 61,175.3 gm. per square centimeter, or considerably more than the average, 40,731.3 gm., required for the cortex of the tubers which rotted when inoculated in that region. All the tubers of the Green Mountain variety rotted. All the Bliss Triumph tubers, except two, became infected from cortical inoculations. These two required a somewhat higher pressure to puncture the tissue of the cortex than the average for this region, but the difference was not great. There is evidently a correlation between the resistance of the tuber to puncture and resistance to infection by the fungus.

The difference in resistance to puncture by mechanical means in these three varieties of potatoes is very marked; and it was considered of interest to see if there was correlated with it some variation in the chemical composition of the tubers, especially in the constituents of the cell wall. Accordingly, determinations of the pentosan and crude fiber content of the two regions, cortex and central portion, of potatoes of each of the three varieties were made.

In preparing the samples for analysis the potato was cut into slices about 8 mm. in thickness, a thin peeling removed with a sharp knife, and the cortex sliced away. This was dried, ground, and analyzed. The central portion of the potato after the ring of bundles had been peeled off was treated in like manner. Pentosan and crude-fiber analyses were made and were calculated to dry weight, the dry weights being obtained in the usual way by drying to constant weight in a vacuum oven. Duplicate determinations were made. The data obtained from the analyses are given in Tables VIII and IX.

TABLE VIII.—*Pentosan content of cortex and interior tissue of potatoes*

[Expressed as percentage of dry weight]

Location of tissue.	McCormick.	Green Mountain.	Bliss Triumph.
Cortex	2.00	1.60	1.86
	2.23	1.70	1.74
Interior	1.40	1.60	1.71
	1.32	1.70	1.65

TABLE IX.—*Crude-fiber content of cortex and interior tissue of McCormick, Bliss Triumph, and Green Mountain potatoes*

[Expressed as percentage of dry weight]

Location of tissue.	McCormick.	Green Mountain.	Bliss Triumph.
Cortex	3.42	2.01	1.98
	3.12	1.93	1.95
Interior	2.12	1.96	1.88
	2.18	1.83	1.92

From Table VIII it is evident that the pentosan content of the cortex of McCormick potatoes is somewhat higher than that for the other two varieties. The pentosan content of the central portion, however, seems to be somewhat lower in this variety. In Table IX is shown the crude-fiber content of the three varieties. McCormicks are higher in crude-fiber content than either of the other varieties. In the cortex of the McCormicks there is over 50 per cent more crude fiber than in the same region of the other two varieties. The interior also of the McCormick tubers

is higher in crude-fiber content than the cortex of Green Mountain or of Bliss Triumph. There is evidently correlated with resistance to mechanical puncture and resistance to infection by *Pythium debaryanum* a higher crude-fiber content.

Further evidence that the resistance of potatoes to infection was correlated with resistance of the tissue to mechanical puncture was obtained from experiments in which Bliss Triumph and Green Mountain tubers were prepared for inoculation by scooping out small plugs of tissue from the cortex of the tubers and allowing the wounds to dry for a given length of time. The plugs were removed by means of a small curved knife, leaving a cavity in the cortex of the tuber about 4 mm. in diameter and of the same depth, without sharp corners or rough surfaces. Part of these potatoes were inoculated as controls, and all of them were placed in the incubator at 30° C. At 3-hour intervals for 12 hours a number of the uninoculated tubers were removed from the incubator, inoculated in the cavities made at the beginning of the experiment, and replaced in the incubator. The inoculations were made in the usual way by placing a bit of mycelium in a drop or two of sterilized water in the cavity, which was then inclosed in a covered Van Tieghm cell. When at the end of 4 days the tubers were removed from the incubator and examined, it was found that only the controls inoculated when the experiment was set up had rotted. None of the wounded potatoes inoculated 3 hours or more after they had been placed in the incubator were rotting. Apparently exposure to the air at 30° C. for 3 hours was sufficient time for the formation of a layer over the wound resistant to fungus attack.

It is commonly considered by the potato growers of the San Joaquin Valley, Calif., that wounds which have had opportunity to cork over will not become infected. This has been shown to be true in these studies by many unsuccessful attempts to inoculate tubers in old wounds. From the experiments described in this paper it is evident that the protective covering is formed very quickly under the conditions of the experiment. Appel(1) claims that the tissue of some varieties of potatoes begins to cork over in 6 hours. That a protective covering is formed in 3 hours under the conditions of the experiment is evident. There is, however, no evidence that it is a true suberization.

The pressure necessary to puncture the tissue of these Green Mountain and Bliss Triumph potatoes was determined on freshly cut slices of the tubers and on slices which had remained in the incubator for 3 hours. The results are shown in Table X.

TABLE X.—*Pressure in grams per square centimeter required to puncture tissue in slices of potatoes freshly cut and after drying for 3 hours at 30° C.*

GREEN MOUNTAIN

Potato No.	Cortex.			Interior.		
	Fresh surface.	Dried 3 hours.	Average increase in pressure required for puncturing dried tissue.	Fresh surface.	Dried 3 hours.	Average increase in pressure required for puncturing dried tissue.
1	40,852.1	59,295.1		30,554.8	58,388	
2	47,738.9	72,409.8		30,554.3	52,737.8	
3	39,553.2	68,851.9		27,528.2	57,551	
4	43,112	76,385.9		31,810	70,344.8	
5	27,833.7	74,711.7		32,019.2	48,552	
Average	39,017.7	70,316.8	31,299.1	29,493.3	57,574.7	28,021.4

BLISS TRIUMPH

1	42,528.3	76,176.8		29,904.8	48,113.6	
2	47,715	79,315.9		27,024.4	46,259.1	
3	41,018.1	64,450		22,601.9	78,068	
4	38,448.3	57,330.6		33,605.6	49,644.2	
5	35,527	60,090.1		26,366.8	57,715	
Average	41,057.3	67,553.2	26,496.9	27,931.1	55,962.1	28,031.0

From the results shown in Table X it is evident that the resistance of the wounded surface of the potato to puncture is appreciably increased in every instance by exposure to the air for 3 hours. In the five Green Mountain potatoes the average increase in pressure required to puncture the cortical tissue was 79 per cent, and the average increase for the central tissue was 95 per cent. With the same number of Bliss Triumph tubers the results were 64 per cent and 100 per cent for the cortex and central portions, respectively.

There is then correlated with the resistance to infection shown by wounds after 3 hours' drying a very marked resistance to mechanical puncture. If the fungus penetrates the tissue mechanically, it is quite possible this increase in resistance, due to drying, would be sufficient to prevent its entrance. It is noticeable that the pressure required to puncture the dried cortex, the region in which the inoculations were made in these experiments, most closely approaches the averages for the inner portion of McCormick tubers which did not become infected.

Another point which is of interest in this connection is the fact that no cases of natural infection through the potato skin have been observed, and repeated attempts in the laboratory to inoculate tubers on the surface have yielded negative results. Correlated with this resistance to infection is a very marked resistance to mechanical puncture. It was exceedingly difficult to puncture the skin of the potato with the round-

tipped glass needles, and the pressure required was considerably more than that required for any portion of the cut surface of tubers tested.

No direct evidence was obtained that the fungus could exert sufficient pressure upon the cell walls of susceptible potato tubers to puncture them. However, some indirect measurements were made of the pressure the fungus might be capable of exerting under certain conditions. When fungus filaments were plasmolyzed in cane sugar solution it was found that it required a solution capable of exerting about 54 atmospheres, or 55,773.3 gm., per square centimeter to plasmolyze them. If, then, the protoplasm of the fungus is not permeable to cane sugar, the filaments are capable of withstanding nearly 55,773.3 gm. pressure per square centimeter; or, stated in another way, the filaments are capable of exerting that much pressure. This is considerably more pressure than is required to puncture the tissue of the central parts of Bliss Triumph and Green Mountain potatoes. It is sufficient pressure to puncture the cell walls of the cortex of all tubers of these varieties which rotted when inoculated in that region except one. This exception is tuber 31 in Table V, a Green Mountain tuber which required 57,571.5 gm. per square centimeter, or 1,798.2 gm. more than the osmotic pressure of the fungus filament as found in this study. It is also sufficient to puncture the tissue of the two McCormick tubers which rotted when inoculated in the central portion and the one which rotted when inoculated in the cortex.

The pressure would not be sufficient to puncture the cell walls of McCormick tubers when they were resistant to infection, and it is lower than that required for the cortex of two of the three Green Mountain tubers that did not rot when inoculated in that region. The third Green Mountain and the two Bliss Triumph tubers that did not rot when inoculated in the cortex required pressures considerably below the osmotic pressure of the fungus filament to puncture the cell walls. Just why these three potatoes did not rot is not apparent. It is, of course, possible that the 10 determinations of the pressure required to puncture cells of the cortex were made on less resistant cells than those upon which the inoculations were made. Another possibility is that a weak culture of the fungus was used. These 3 potatoes, however, were exceptions to the rule.

The experiments for the determination of the pressure required to puncture the tissue of the potatoes on the fresh surface and when dried at 30° C., as detailed in Table X, show that the pressures required for the cortex of the dried tubers, which were resistant to infection, were considerably higher than the osmotic pressure of the fungus filament. This is in agreement with the evidence just brought out from data in Tables V to VII. It would seem from this work that the mechanical pressure of the fungus filament against the cell wall of the potato is an important factor in the penetration of the potato tissue by the fungus.

One consideration detracts from the value of this indirect method for the determination of the pressure the fungus filament is able to exert against the cell walls. In the osmotic pressure determinations, an attempt was made to determine the total pressures within the filaments of the fungus, and these may or may not be the pressure the fungus is able to exert against the cell wall of its host plant. The cell walls of the fungus filament are apparently able under ordinary conditions to withstand the pressure within the filament, except at the growing points. The pressure exerted on the cell wall of the potato under the most ideal conditions would be the pressure that the contents of the filaments

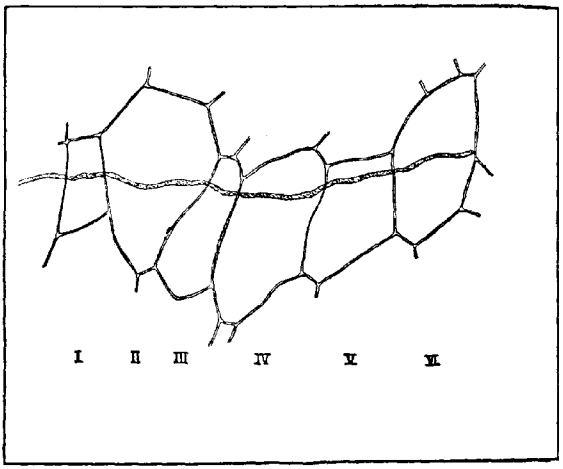

FIG. 1.—Drawing to illustrate growth of a Pythium hypha in potato tissue. Note the constriction of the hypha where it penetrates the wall.

were capable of exerting minus the pressure necessary to push out the wall or rudimentary wall of the tip of the fungus filament.

Further evidence on the method by which the fungus penetrated the cells of the potato was furnished by direct observations of the hyphae of the fungus within the tissue of the potato. In these experiments sections of raw potato were prepared as nearly sterile as possible and inoculated with the fungus. When kept overnight in hanging drop cells at 30° C. a good growth of hyphae was usually obtained. Numerous instances of cell-wall penetration were observed, and the method of penetration was followed both by serial drawings and by motion photomicrographs (Pl. 36, 37). The part of the section selected for observa-

tion was usually two or three cells thick, since if the section is much thinner the hyphae are liable to grow over the surface.

The hypha shown in figure 1 was watched continuously for three hours. During this time it grew 1,976 μ at a room temperature of about 70° F. The time required to penetrate the wall was about 5 minutes. The distance traversed and the time required for each cell were as follows:

The hypha in cell I, traversed 200.3 microns in 25 minutes.
The hypha in cell II, traversed 493.9 microns in 35 minutes.
The hypha in cell III, traversed 186.9 microns in 20 minutes.
The hypha in cell IV, traversed 387.2 microns in 20 minutes.
The hypha in cell V, traversed 333.7 microns in 20 minutes.
The hypha in cell VI, traversed 373.8 microns in 30 minutes.

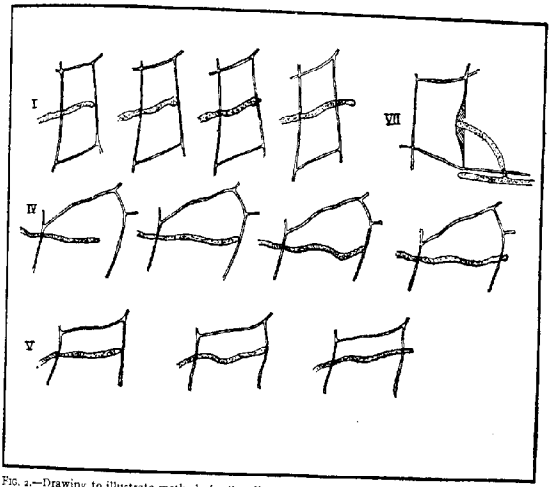

FIG. 2.—Drawing to illustrate method of cell-wall penetration in cells I, IV, and V. For explanation see text.

The drawings of figure 2, made at the time of observation to show the relative positions assumed, give the characteristic methods of cell-wall penetration as observed in this study. In passing through the cell wall between cells I and II the hypha approached the cell wall nearly at right angles; it formed a swelling at the end, bent slightly, and penetrated the wall by a small tube. After passing through the cell wall into the next cell the hypha expanded to its usual diameter. Considerably more bending of the hypha is shown in cells IV and V. It is noticeable that the wall in cell V bends outward under the pressure of the hypha and that the hypha straightens after the wall is penetrated.

There is quite clear evidence of the exertion of mechanical pressure. In cell VII another hypha was observed growing toward the potato cell wall. At first the hypha was straight; then as growth pressed it against the wall it bent upward, at the same time making a dent in the wall near the center of the wall face. This hypha did not penetrate the wall, for when it had reached the position given in the figure, rapid growth was begun by the growing tip just below it, and the pressure of the upper hypha seemed to be insufficient to break the wall.

Where the hypha approaches the cell wall at right angles it usually passes through as shown in cell I; but when the tip strikes the wall obliquely it does not usually penetrate but pushes along the wall and may go entirely around the cell, forming a coil within it. In traveling around the cell the tip may reach a corner, in which case the wall is frequently penetrated and the hypha grows between the cells. This is characteristic, and hyphae are frequently found following the middle lamellae. When the growth of the young tip is stopped for a few moments as by strong light, a cell wall is apparently formed over the tip; and on the resumption of growth this wall is broken at its weakest point by the pressure developed within the hypha. The formation of a strong hyphal wall requires about two minutes under the conditions of these experiments. The swelling of the tip shown in cell I took place in about two minutes—that is, the cell wall of the hypha seemed to become strong enough in that length of time to withstand the pressure within. There must, then, be a rapid transformation of the fluid protoplasmic material to form this wall. This transformation may be of the nature of a precipitation at the boundary between the hyphal sap and the potato cell sap. It is possible that the precipitation of substances to form the strong hyphal wall occurs only in contact with the cell sap of the potato, at least occurs more rapidly in contact with the potato cell sap than in contact with the cell wall. If this is true the hypha would form a tube of plastic materials against the cell wall and the growth pressure of the fungus filament would be applied directly to the cell wall of the potato. This might be the explanation of the mechanics of cell-wall penetration by the fungus. Evidence that the hyphae are sometimes cemented fast to the cell wall was secured during observations of hyphae that strike the cell wall obliquely, slide along it for a short distance, and then stop and penetrate the wall.

DISCUSSION OF RESULTS

In a consideration of the results brought out in the foregoing pages it is apparent that there is much evidence that the fungus makes its way through the cell walls of the potato mechanically. It is, of course, impossible to prove in work of this kind that some enzym is not secreted at the tip of the hypha which softens or destroys the portion of the cell wall with which it is directly in contact. If, then, no evidence of the

existence of such an enzym was brought out in this study, the fact that the fungus requires such a short time (about five minutes) to pass completely through a cell wall seems to indicate that the main factor, at least in the breaking through the cell wall, is mechanical pressure, for in enzym action it would be necessary to have a diffusion of the enzym from the tip of the hypha into the cell wall and at least a softening, if not a dissolving, of a portion of the cell wall at that point. Whether a substance, such as an enzym, with a relatively high molecular weight and consequently low rate of diffusion could diffuse into and through the cell wall with sufficient rapidity to soften or dissolve this tissue in the time required for the fungus to pass through the cell wall is doubtful. Another point in support of the hypothesis that the opening in the cell wall is made mechanically is that there is apparently no considerable increase in the size of the opening after the tip of the hypha passes through. If the fungus secretes an enzym which acts on the cell wall, it would seem probable that this enzym action would continue after the tip of the hypha passed through and the opening would be larger than the hypha. Hasselbring (*13*) has figured the breaking down of the host tissue around the fungus hypha. This phenomenon is common where a fungus breaks down the cell walls of its host enzymically. With *Pythium debaryanum* on potato, however, the opening in the cell wall is never larger than the mean diameter of the hypha in the lumen of the cells and is usually considerably smaller.

Another point which supports the hypothesis of the mechanical puncture of the cell walls of the host by the fungus is the fact that apparently only the middle lamella of the potato cell wall is affected. The fungus seems not to break down the secondary thickening of the cell walls, and when a piece of well-rotted potato is teased out on a slide the cells full of starch grains float free.

If, then, as seems probable, the fungus makes its way through the cell walls by mechanically puncturing them, a potato with cell walls strong enough to withstand the pressure exerted by the fungus would be immune to the disease. If we consider the osmotic pressure within the fungus filament—as determined in this study—as the pressure the fungus is able to exert against the wall of its host plant, then the resistance of potatoes in all cases in which they did not become infected would be explained, with the three exceptions mentioned earlier. This would also account for the infection of all potatoes which became infected in either the central portion of the tuber or the cortex, with the exception of one Green Mountain tuber. Another point which should be noted in this connection is that correlated with this resistance is a higher crude-fiber content in the McCormick. This is probably due to more secondary thickening in the cell walls. It is quite possible that the White McCormick potato or some hybrid of this variety would be resistant to the fungus when grown in the San Joaquin Valley and would thus solve

the problem of the control of this disease, but no field tests have been made for varietal susceptibility.

If the fungus enters the potato cell by breaking through the cell walls mechanically, it is of course necessary that there be some support from which this pressure may be developed. This support would be readily furnished where the fungus filaments were against the opposite cell wall—for instance, when the fungus is within the tissue. It seems probable also, as has been brought out earlier in this paper, that the fungus may attach itself to the cell walls of its host. In the penetration of the host tissue from the outside, as it took place in this study, it is of course necessary to have an attachment of the fungus hyphae to the host tissue. This may be accomplished by the newly formed wall of the fungus hyphae adhering to the cell wall of the potato. Blackman and Welsford (5) considered that the mucilaginous membrane of the germ tube of Botrytis formed an attachment sufficiently strong to withstand the pressure necessary for the puncturing of the cuticle of broad bean leaves. In natural infections the fungus hyphae are frequently thrust deep into the tissues of the potato, and a support from which the pressure could be developed would readily be found by the closing of the wound in the tuber.

If in its growth in the potato this fungus breaks its way through the tissue mainly by mechanical means, as seems quite possible, it is in keeping with the manner in which roots grow through potato tissue. Peirce (24) has shown that roots of *Pisum* sp. and *Vicia faba* can force their way through potato tissue mechanically, and one of the present writers has frequently observed potatoes in the San Joaquin Valley with roots growing through them. A somewhat analagous condition is found in the penetration of the stigma and style of certain Rubiaceae by the pollen tube, as described by Lloyd (19).

While it has not been proved in this investigation that *Pythium debaryanum* penetrates the cell walls of the potato by mechanical pressure, there is considerable evidence that the main factor in this penetration is the growth pressure of the fungus filament and that the resistance of the White McCormick potatoes to this disease is due to cell walls that are more resistant to mechanical puncture than are the cell walls of extremely susceptible varieties.

SUMMARY

(1) It has been shown in this paper that *Pythium debaryanum* destroys the pentosans, starch, and sugar of the potato tuber in rotting it.

(2) The fungus secretes a toxin which kills the cells of the potato. It also secretes an enzym which breaks down the middle lamellae of the cells but apparently has little or no effect on the secondary thickening.

(3) More pressure was required to puncture the tissues of White McCormick potatoes, which are comparatively resistant to the disease,

than to puncture the tissues of the two susceptible varieties, Bliss Triumph and Green Mountain. Correlated with this resistance to puncture is a resistance to infection by *Pythium debaryanum*.

(4) The resistance to puncture in McCormick tubers is also correlated with a higher crude-fiber content, which was considered to be due to more secondary thickening in the cell walls.

(5) The cut surface of the cortex of Bliss Triumph and Green Mountain when dried for three hours was much more resistant to puncture than the freshly cut surface. Here also there is a correlation between resistance to infection by this fungus and resistance to mechanical puncture.

(6) The osmotic pressure within the fungus filament, as determined by plasmolysis in this work, was sufficient to develop the pressure necessary to puncture the cell walls in the potato tubers in all cases in which infection occurred, with one exception. It was not sufficient to develop the pressure necessary to puncture the tissue of the potatoes in the cases where no infection occurred, with three exceptions.

(7) Mechanical pressure exerted by the fungus hyphae seems to be the most important factor in cell-wall penetration by this fungus, and resistance to infection is apparently due to resistance of the cell walls to mechanical puncture. Microscopical observations of cell wall penetration by the fungus hyphae seem to corroborate this theory.

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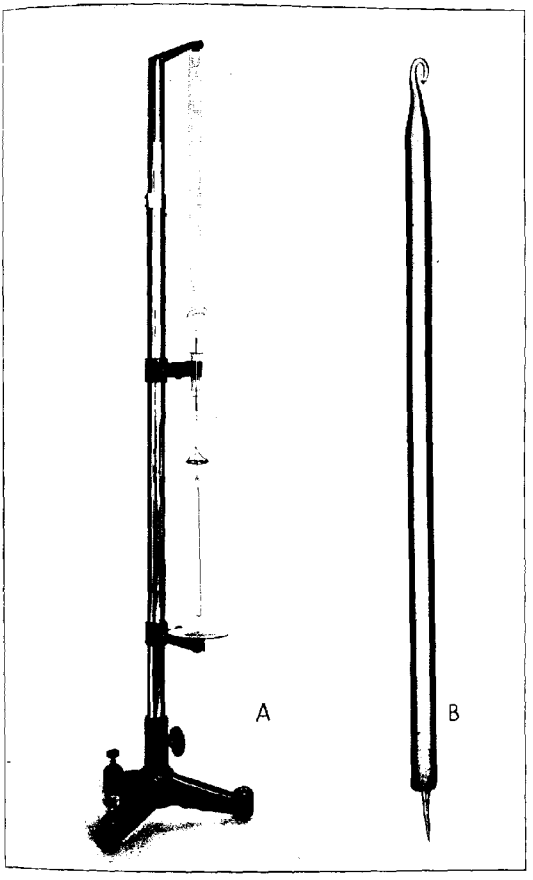
PLATE 35

A.—Apparatus used in determining the pressure required to puncture the tissue of potato tubers.

B.—Glass rod with attached needle. About actual size. Photographs by J. F. Brewer.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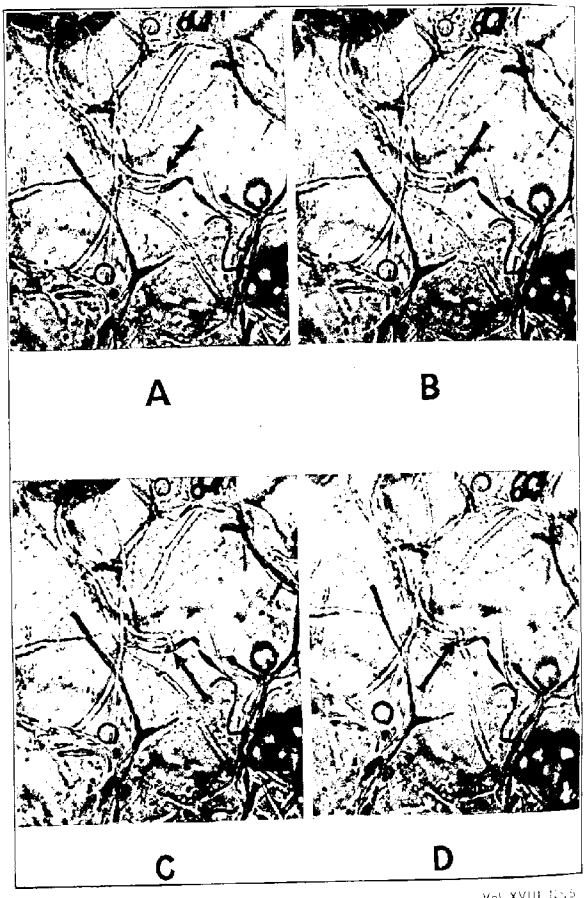
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PLATE 36

Photographs enlarged from portions of a motion photomicrograph, showing the method of cell wall penetration by Pythium hyphae.

A.—Shows hypha growing toward the potato cell wall.

B.—Shows hypha attached to wall and about to penetrate.

C.—The tip has just broken through the wall.

D.—The penetration is complete. Note the black line at the point where the hypha penetrates the wall. This may be due to a rolling up of the potato cell wall about the hypha or to a difference in refraction caused by compression of the wall.

PLATE 37

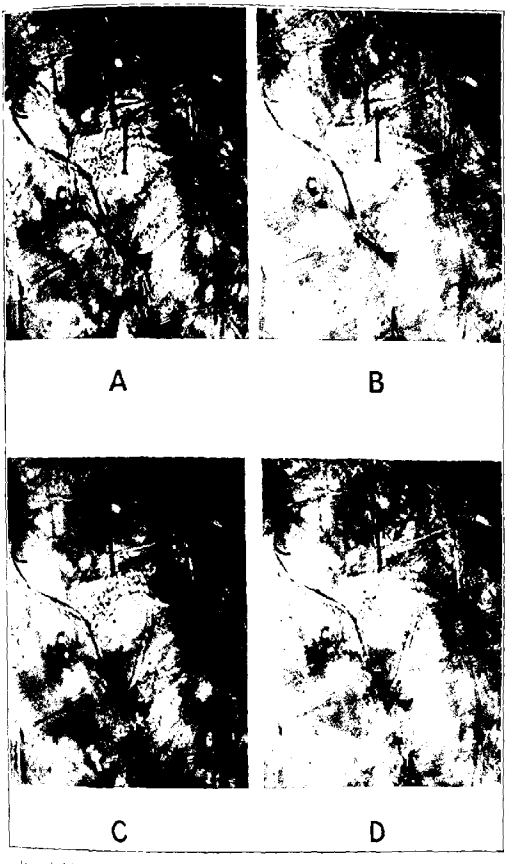
Photographs enlarged from portions of a motion photomicrograph, showing the method of cell wall penetration by Pythium hyphae.

A.—Shows the hypha growing against the potato cell wall. Sufficient pressure has already been applied to cause the hypha to bend. Notice that this bending increases in later photographs.

B.—A little later stage than A.

C.—The tip has broken through as a small tube.

D.—Penetration is complete. Notice the constriction of the hypha at the point where it penetrates the potato cell wall.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LOSSES OF ORGANIC MATTER IN MAKING BROWN AND BLACK ALFALFA[1]

By C. O. SWANSON, L. E. CALL, and S. C. SALMON, *Kansas Agricultural Experiment Station*

Large losses of alfalfa[2] due to improper curing of the first crop have led to the employment of methods other than that of curing in the field and stacking. Some farmers convert the green alfalfa into silage, but there are so many difficulties[3] in making good silage from alfalfa that this method is rarely practised. Others stack the alfalfa in a partially wilted condition. The great weight excludes the air, and fermentations occur somewhat similar to those which occur in a silo. The product is known as brown and black alfalfa. The degree of color depends upon the conditions which control the nature and extent of the fermentations. Some of these conditions are moisture content of the alfalfa when stacked, size and shape of the stack, and temperature and rainfall during the time of curing. Such alfalfa, according to growers who use this method, is relished by cattle; and some practical feeders consider it superior to ordinary alfalfa hay.

However, when fermentation occurs there is evidently a loss in nutritive value. Since the nature and amount of these losses apparently were unknown, the writers decided to investigate them and also to compare the feeding value of black and brown alfalfa with that cured in the usual way.

For the purpose of this experiment a uniform field of alfalfa, estimated to make a 45- to 50-ton stack, was selected. The alfalfa was cut, wilted for a few hours and stacked in the open, each load being weighed separately. Some wilting was considered necessary in order to get a desirable product and also because hay loaders will not work satisfactorily in unwilted alfalfa.

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[1] Contribution from the Department of Agronomy (paper No. 16) and the Department of Chemistry of the Agricultural Experiment Station of the Kansas State Agricultural College. The Department of Animal Husbandry conducted the feeding trials. The chemical work was done in the analytical laboratory in charge of Assistant Professor W. L. Latshaw.

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Samples of 10 to 20 pounds each were taken from the different loads as the alfalfa was hauled to the stack. These were placed in bags and sent at once to the chemical laboratory. Here they were weighed again and the contents removed from the sack and spread out to dry. Care was taken to prevent any loss. When the samples were air-dry they were weighed again and passed through a feed cutter, and the moisture in the air-dry material was determined. The total moisture in the original samples was then calculated and was found to vary from 29 to 70 per cent, the average being 53.28 per cent. The percentage of feed constituents in the dry material was as follows: Ash, 9.27; protein, 17.25; crude fiber, 38.97; and ether extract, 2.68.

A few samples of the freshly cut alfalfa were also taken. The moisture content of these varied from 70 to 77 per cent and averaged 72.1 per cent. These variations illustrate some of the difficulties of conducting the experiment and should be considered in interpreting the results. The range in moisture content can be seen from the percentages given in Table 1.

TABLE I.—*Percentage of moisture and dry matter in samples taken at time of stacking*

Sample No.	Description.	Total moisture.	Dry matter.
359	Alfalfa ready to load	69.36	30.64
360	do	71.05	28.95
362	do	66.94	33.06
365	do	52.43	47.57
366	do	53.21	46.79
368	do	57.55	42.45
373	do	29.25	70.75
374	do	38.68	61.32
381	do	50.52	49.48
382	do	41.06	58.94
386	do	56.04	43.96
361	Alfalfa just cut	77.22	22.78
364	do	71.55	28.45
363	do	75.22	24.78
367	do	71.20	28.80
385	do	69.49	30.51
	Average of all	59.42	40.58
	Average of "ready to load"	53.28	46.72

The alfalfa remained in the stack till early winter, when the stack was measured into four quarters. The plan was to leave one quarter intact until early spring. The alfalfa from the three other quarters was used in a feeding experiment with steers in which the black alfalfa from this stack was compared with good quality green alfalfa and also good quality brown alfalfa. Three samples were taken the latter part of December from the material and fed to steers. The last quarter was loaded and weighed the last part of March, and at which time the different kinds of

alfalfa present in this last quarter were sampled. It was assumed that the last quarter represented the whole stack, so the weights of the different kinds of hay removed from the stack were multiplied by four to obtain the weight of each kind of hay in the whole stack.

The total weight of hay removed from the stack, the estimated amounts of each kind, and the analysis of each kind, based on the samples taken the last of March from the last fourth of the stack, are given in Tables II and III.

TABLE II.—*Composition of samples of brown and black alfalfa taken from the stack*

Sample No.	Date of sampling.	Description of sample.	Total moisture.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
			Per ct.	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*	*Per ct.*
581	Dec. 28, 1916	Black alfalfa, charred, inferior quality	70.39	3.99	5.55	11.91	7.66	0.50
582	do	do	61.84	6.35	7.37	11.21	12.37	.86
591	do	Black alfalfa, good quality	63.40	4.60	6.57	10.63	14.00	.79
592	Mar. 20, 1917	do	59.28	5.79	7.44	11.94	15.57	1.08
600	Mar. 28, 1917	Charred dry, not moldy	15.70	13.50	10.61	23.29	29.12	1.72
601	do	Partly moldy, but moist, second grade	48.54	13.40	10.53	12.83	13.81	.76
602	do	Black alfalfa, good quality	52.45	7.40	9.06	15.62	14.52	1.03
603	do	From stack bottom, bad odor	65.80	6.71	4.62	11.39	10.39	.99
604	do	Alfalfa hay, color and odor good	58.17	5.94	6.84	14.66	13.13	1.10
605	do	Dark brown hay, next to charred portion	5.73	13.62	17.53	21.47	40.24	1.56
606	do	Moldy, mostly charred	32.99	14.33	17.81	12.84	21.89	.87
607	do	Green hay from outside of stack, good	5.02	10.39	13.65	31.99	37.95	.73
608	do	Light brown hay	3.80	14.20	16.39	28.29	36.56	.91

TABLE III.—*Weight of dry matter and chemical constituents of brown and black alfalfa taken from the stack, compared with the amounts put into the stack*

Sample No.	Description of sample.	Hay.	Dry matter.	Ash.	Protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.
		Lbs.	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*	*Lbs.*
600	Charred hay	20,440	17,231	2,759	3,401	4,760	5,952	358
601	Partly moldy hay	8,040	4,137	1,077	846	1,032	1,110	61
602	Black hay	9,020	4,574	711	872	1,503	1,397	99
603	Stack bottom, bad odor	1,680	575	112	78	191	175	17
604	Green hay (?)	6,000	2,520	356	410	880	788	66
605	Dark brown hay	5,440	5,128	741	954	1,168	2,184	85
606	Moldy and charred (?) on top of stack	5,400	3,619	774	962	695	1,182	5
607	Green hay from the outside, good	4,720	3,913	428	562	1,317	1,564	30
608	Light brown hay	7,400	7,119	1,051	1,213	2,087	2,705	67
	Total taken from stack	68,140	48,806	8,009	9,298	13,630	17,057	782
	Original hay as put into the stack	171,480	80,115	7,426	13,819	25,500	31,720	2,147
	Loss	31,309	a 583	4,521	12,870	14,193	1,356
	Percentage of loss	39.07	a 7.84	32.71	46.54	45.36	63.57

a Coin. This gain of ash is not large considering the nature of the experiment and the assumption made in the calculations.

LOSSES OF ORGANIC MATTER

The total weight of the partially wilted alfalfa put into the stack was 171,480 pounds, of which 80,115 pounds were dry matter as determined by the average dry-matter content of all samples, which was 46.72 per cent.

The weights of the different kinds of material removed from the stack (Table II) totaled 68,140 pounds, of which 48,806 pounds were dry